



# Multiple roles of perlecan in cartilage development, wound healing and tumor metastasis

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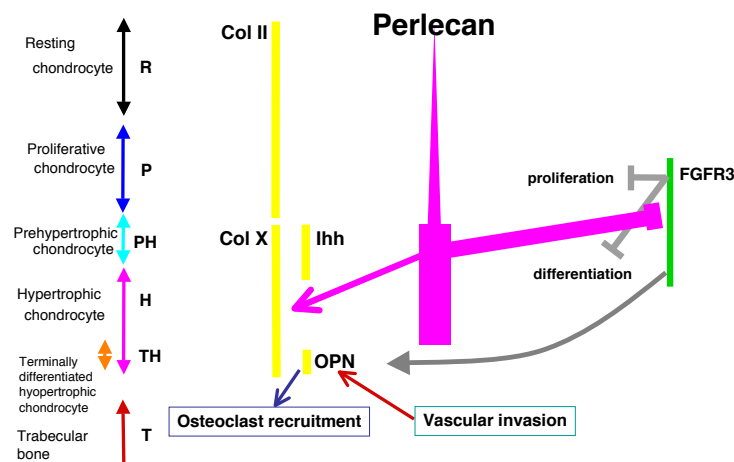
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After I received my Ph.D. in biology from Osaka University in Japan in 1971, I worked as a postdoctoral fellow at the Department of Biochemistry, University of Pittsburgh Medical School, where I studied the gene regulation of bacteriophages and plasmids. In 1979, I joined Dr. Benoit deCrombrughe's group at the National Cancer Institute, NIH, where I started cloning collagen genes (e.g., collagen 1, II and III) and studying their gene structure, regulation and evolution. In 1983,

I moved to the National Institute of Dental and Craniofacial Research (NIDCR), which is also on the NIH campus in Bethesda, where I have extended my research field to other matrix genes. In my early career at NIDCR, I cloned and characterized many extracellular genes, including collagen IV, laminins, perlecan, aggrecan, and link protein. I also studied the structure and function relationships of these gene products. More recently, I have been working on tissue development and functions in animal models and diseases for these matrix proteins. I have also identified novel genes important for tooth and craniofacial development and studied their functions in mouse models, cell cultures, and diseases.

## Multiple functions of perlecan in endochondral ossification



Perlecan is a major heparan sulfate proteoglycan in basement membrane and in some other tissues such as cartilage. The protein core (400 kDa) of perlecan consists of five distinct domains that can be substituted primarily with heparan sulfate (HS) chains. Perlecan interacts with many extracellular

molecules and cell surface receptors through HS chains and the protein core which contribute to strengthening the basement membrane structure and serving as a multifaceted functional proteoglycan. Studies with gene knockout mice and human genetic diseases demonstrated that perlecan is essential for

development and that the lack of perlecan results in either embryonic lethality due to defective myocardial basement membrane or perinatal lethality due to cartilage defects. In addition, perlecan is implicated in many biological functions in tissue homeostasis and diseases. In this presentation, I will focus on the roles of perlecan in cartilage development, tumor metastasis and skin wound repair using animal models.

Perlecan KO mice develop perinatal lethal chondrodysplasia. Histological evaluation of the perlecan KO mice revealed a disorganized growth plate with reduced chondrocyte proliferation and differentiation and defective endochondral ossification. The columnar structure of the hypertrophic chondrocytes is severely disrupted in the mutant growth plate. The abnormalities of cartilage are not obvious until approximately E14.5, at which time endochondral ossification begins, and after that, disruption of cartilage matrix becomes progressively severe. We propose two functions of perlecan in normal cartilage development: 1. modulation of growth factor activity, such as FGF/FGFR3c, and 2. formation of the extracellular matrix in the hypertrophic zone. FGFR3c, the FGF receptor specific to chondrocytes, regulates cartilage development by inhibiting chondrocyte proliferation and expression of Indian hedgehog (IHH). Activating mutations of FGFR3c, which cause Thanatophoric dysplasia, the most common human lethal chondrodysplasia, result in a reduced proliferative zone and a shortened growth plate, similar to perlecan-null mice, whereas Fgfr3-null mice develop an opposite phenotype, i.e., expansion of the proliferative and hypertrophic zones and survival. To test the modulation of Fgfr3c activity by perlecan, we used limb organ cultures from KO mice and Fgfr inhibitors. We also created double-KO mice for perlecan and Fgfr3 to examine if the abnormal phenotypes of perlecan KO cartilage can be restored

in the absence of Fgfr3. Our data indicated that the inhibition of Fgfr in the growth plate cartilage of KO mice increased chondrocyte proliferation and restored the Ihh-expressing prehypertrophic chondrocyte zone, but failed to differentiate into hypertrophic chondrocytes. These results suggest that in normal cartilage development, perlecan inhibits Fgfr3c activity probably by trapping Fgf in the matrix. The modulation of Fgfr3c activity by perlecan allows the appropriate size and expansion of the growth plate. Further, perlecan plays a critical role in matrix formation in the hypertrophic zone. Without the matrix, cells fail to form an organized columnar structure and cannot differentiate well. Since perlecan is predominantly located in the pericellular space of hypertrophic chondrocytes, it is possible that perlecan interacts with both ECM and a cell surface receptor and stabilizes the interaction of ECM and cells to form organized columnar cell structure in the hypertrophic zone.

Animal models are useful to determine the roles of perlecan in adult tissue function and disease. However, the lethal phenotype of perlecan KO mice has hampered these studies. To overcome the problem, we created perinatal lethality-rescued perlecan KO mice by expressing recombinant perlecan specifically to cartilage but not other tissues. The mutant mice survived but had small eyes and a myotonia phenotype, similar to patients with partial functional mutations in perlecan. Using the mutant mice, we found that in the absence of perlecan, tumor metastasis was decreased in an experimental metastasis model of B16-F10 melanoma cells. We also found that skin wound healing was accelerated and cell migration of keratinocytes and fibroblasts was increased in the absence of perlecan. Although the mechanisms of these phenotypes are not clear, our results suggest that perlecan plays important roles in cellular processes in various tissues in adults.

**Keywords :** Heparan sulfate proteoglycan, Perlecan, Cartilage development, FGFR, Tumor metastasis, Wound repair