Heparan sulfate proteoglycans localize in the extracellular matrix and on the external surface of cell membranes, and play important roles in cell-cell and cell-extracellular matrix interactions. Heparanase is an endo-beta-D-glucuronidase capable of cleaving heparan sulfate and has been implicated in inflammation, angiogenesis and tumor metastasis. Inhibition of heparanase activity may reduce metastasis of cancer cells, as degradation of the extracellular matrix is crucial for allowing tumor cells to penetrate tissue barriers and metastasize.

In this study, we established a stable heparanase expression system by cloning heparanase cDNA from a human placenta cDNA library. During the course of cloning, we obtained two cDNAs: a 1.7kb cDNA which is a known heparanase cDNA, and a 1.5kb cDNA, which is thought to be a deletion of exon5, as revealed by sequencing. To investigate the regulation of heparanase activity, we transfected the 1.7kb cDNA and the 1.5kb cDNA into COS-7 cells. The activity was measured by the degradation products of heparan sulfate. When the 1.7kb cDNA was expressed, high heparanase activity was observed. On the other hand, no heparanase activity was observed when the 1.5kb cDNA was expressed.

Experiments using tunicamycin A and several endoglycosidases showed that oligosaccharide chains in heparanase and its deleted form are high mannose type. Reverse transcription polymerase chain reaction experiments revealed that heparanase was expressed in the placenta, ovary, lung, heart and fetal brain. The splicing variant, although far less than normal form, was also expressed in the entire tissues examined.

Subcellular localization of heparanase and its alternative splicing form in the transfectants was examined by immunofluorescence staining, and the results indicate that heparanase is localized within endoplasmic reticulum. No differences in their intracellular localization were observed between heparanase and its splicing variant. Considering the function of heparanase working outside the cells, the localization of heparanase is a major determinant in regulating the biological functions. If the splicing or localization of heparanase could be regulated, it may be helpful in reducing cancer cell metastasis.

BMPs stimulate osteoclastic boneresorption and remodeling during endochondral bone development and fracture repair

During endochondral bone development, cartilage anlagen is initially formed and replaced by bone. Bone is further remodeled by osteoclastic resorption coupled with osteoblastic formation. In fracture repair, this developmental process is recapitulated. Bone morphogenetic proteins (BMPs) are secreted molecules capable of inducing ectopic endochondral ossification and thus have been expected to enhance fracture healing. However, their clinical use in fracture repair has been controversial, suggesting possible complexity in response of bone tissues to exogenous BMPs. Recent osteoblast-specific down-regulation of BMP signals by expression of a dominant-negative form of BMP receptors in mice showed that BMPs induce osteoblast differentiation, whereas several in vitro reports pointed out that BMPs also stimulate osteoclast differentiation directly. Recently, necessary for remodeling bone are unclear. To clarify roles of BMP signaling in endochondral bone development, we first generated transgenic mice overexpressing noggin, an antagonist of BMPs, in bone under the control of the ColIa1 promoter sequence. Unforeseen results were bone thickening in transgenic mice. Rates of bone volume to tissue volume for transgenic mice were 225 % of wild-type. Numbers of osteoclasts marked by TRAP staining were dramatically decreased from 17.5 days postcoitum to 3 weeks after birth. Dynamic bone histomorphometric assays at 3-week-old showed that bone resorption rates decreased significantly (p < 0.05). Bone formation rate was also significantly decreased. In spite of their thickness, transgenic bones were woven and frequently undergo fracture, suggesting insufficient remodeling. In vitro osteoclastogenesis prepared from noggin transgenic mice was normalized by addition of recombinant BMP2 to the medium, suggesting that bone phenotypes of transgenic mice were caused by reduced BMP signaling due to excess noggin. For further clarification of BMP functions, we then generated transgenic mice overexpressing BMP4 during endochondral ossification. BMP4 overexpression in bone caused severe bone loss associated with increased numbers of osteoclasts per bone surface. Meanwhile, overexpression of BMP4 in cartilage expanded cartilage anlagen, leading to large and thick bone in transgenic mice. To investigate whether these phenomena are recapitulated in the process of fracture healing, we injected recombinant BMP2 (rhBMP2) at various stages of healing in the mouse tibia fracture model. Early application of rhBMP2 to the fracture site expanded cartilaginous elements in immature callus, causing enlargement of bony callus which replaced cartilaginous anlagen. On the other hand, late rhBMP2 application caused resorption of bony callus associated with increased number of osteoclasts. Our results collectively suggest that BMPs in bone is not mere an osteoblast inducer, but a stimulator of osteoclasts and bone remodeling necessary for maintaining strength. These findings may contribute to figure out ideal strategy for treating fracture using BMPs. Transient application of BMPs at early phase when cartilage is forming may produce large bony callus that would fill large defects at fracture sites. Prolonged application of large amount of BMPs after bony callus is formed causes continuous bone resorption. Instead, controlled application of small amount of BMPs may regulate bone remodeling, resulting in bone with good mechanical strength.

BMPは内臓骨性骨形成と骨折治癒過程において破骨細胞性骨吸収とリモデリングを促進する

A33 Expression of the heparanase and its alternative splicing variant

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A34 BMPs stimulate osteoclastic boneresorption and remodeling during endochondral bone development and fracture repair

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かん転移促進物質ヘパランーゼのalternative splicingについての研究

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