ing, demonstrated that the *cho* mutation is a single nucleotide deletion located about 570 nucleotides downstream of the 5′ end of α1(XI) collagen mRNA. The deletion causes a shift in reading frame and results in the synthesis of a truncated α1(XI) polypeptide consisting only of the amino-terminal 1/10 of the wild-type protein. Since the levels of *cho* α1(XI) transcripts are much lower than wild-type transcripts, the levels at which such truncated peptides are synthesised must be low. This suggests that the *cho* phenotype is a loss-of-function phenotype.

What are the consequences of the loss of α1(XI) collagen in *cho*? Type XI collagen molecules are heterotrimers of α1(XI), α2(XI), and α3(XI) collagen chains and the absence of α1(XI) must lead to a deficiency of type XI collagen molecules. Collagen fibrils in *cho/cho* cartilage are abnormally thick, thus suggesting that type XI collagen molecules are essential for the formation of a network of thin collagen fibrils in cartilage. This could explain the abnormal extractability of proteoglycans form *cho/cho* cartilage and the lack of cohesiveness, in that fewer, albeit thicker fibrils would provide less of a physical entrapment of large proteoglycan aggregates. Alternatively, type XI collagen molecules or sub-domains may bind to proteoglycans in normal cartilage, and the loss of such binding sites in *cho/cho* cartilage could result in decreased tissue cohesiveness.

What the reasons may be for the defect in chondrocyte differentiation seen in *cho/cho* growth plate regions is not clear. Perhaps type XI collagen molecules along collagen fibrils contain binding sites for chondrocyte matrix receptors that play a role in signalling pathways controlling chondrocyte differentiation. Alternatively, type XI molecules may directly (or indirectly) bind growth factors that are essential for inducing chondrocyte differentiation. Whatever the reasons may be, however, it is clear that the *cho* mice provide a system for studying how extracellular matrix regulates cell differentiation, cellular organisation and tissue cohesiveness. In addition, the mice provide an animal model for studying molecular mechanisms in human disorders due to mutations in type XI collagen chains. The first examples of such disorders are osteochondrodysplasias in two Dutch kindreds linked to the *COL11A2* locus. In one family with an autosomal dominant form of the Stickler syndrome, affected individuals have mild spondyloepiphyseal dysplasia, osteoarthritis, and sensorineural hearing loss[9]. In this family a splice donor site mutation results in 'in-frame' exon skipping within α2(XI) collagen transcripts[10]. In a second family with similar, but more severe characteristics inherited as an autosomal recessive abnormality we have also shown linkage to *COL11A2*[10]. These results suggest that mutations in collagen XI genes are associated with a spectrum of abnormalities in human skeletal development and support the conclusion based on the *cho* studies, that collagen XI is essential for skeletal morphogenesis.

1 Warman, M. L., Abbott, M., Apte, S. S., Hefferon, T., McIntosh, I., Cohn, D., Hecht, J., Olsen, B. R., and Francomano, C.A., Nature Genet. *5* (1993) 79.
2 Shiang, R., Thompson, L. M., Zhu, Y.-Z., Church, D. M., Fiedler, T. J., Bocian, M., Winokur, S. T., and Wasmuth, J. J., Cell *78* (1994) 335.
3 Reardon, W., Winter, R. M., Rutland, P., Pulleyn, L. J., Jones, B. M., and Malcolm, S., Nature Genet. *8* (1994) 98.
4 Hastbacka, J., de la Chapelle, A., Mahtani, M. M., Clines, G., Reeve-Daly, M. P., Daly, M., Hamilton, B. A., Kusumi, K., Trivedi, B., Weaver, A., Coloma, A., Lovett, M., Buckler, A., Kaitila, I., and Lander, E. S., Cell *78* (1994) 1073.
5 Li, Y., Lacerda, D. A., Warman, M. L., Beier, D. R., Yoshioka, H., Ninomiya, Y., Oxford, J. T., Morris, N. P., Andrikopoulos, K., Ramirez, F., Wardell, B. B., Lifferth, G. D., Teuscher, C., Woodward, S. R., Taylor, B. A., Seegmiller, R. E., Olsen, B. R., Cell February 1995.
6 Seegmiller, R. E., Fraser, F. C., and Sheldon, H., J. Cell Biol. *48* (1971) 580.
7 Seegmiller, R. E., Ferguson, C. C., and Sheldon, H., J. Ultrastr. Res. *38* (1972) 288.
8 Monson, C. B., and Seegmiller, R. E., J. Bone Jt Surg. *63*A (1981) 637.
9 Brunner, H. G., van Beersum, S. E. C., Warman, M. L., Olsen, B. R., Ropers, H.-H., and Mariman, E. C. M., Hum. molec. Genet. *3* (1994) 1561.
10 Vikkula, M., Mariman, E. C. M., Lui, V. C. H., Zhidkova, N. I., Tiller, G. E., Goldring, M. B., van Beersum, S. E. C., Malefijt, M., van den Hoogen, F. H. J., Ropers, H.-H., Mayne, R., Cheah, K. S. E., Olsen, B. R., Warman, M. L., and Brunner, H. G., Cell February 1995.

Studies of bone matrix molecules give us insights into bone remodelling

D. Heinegård

Department of Medical and Physiological Chemistry, Lund University, P.O. Box 94, S-221 00 Lund (Sweden), Fax +46(46)113417

Abstract

Bone is one of the most dynamic tissues, with constant remodelling and repair of minor defects during the whole life cycle. These processes have key roles in replacing fatigued, non-functional tissue with fresh tissue which has the proper mechanical properties. In this process, a balance between breakdown of less functional tissue and new tissue production is a prerequisite for tissue integrity and function. One of the major health problems in the elderly, i.e. osteoporosis and

ensuing bone fracture, is caused by an imbalance between breakdown and synthesis in this continuous bone remodelling. Thus the study of cellular and molecular events governing the relevant processes is a prerequisite for understanding mechanisms and for future efficient therapeutical intervention.

One unique feature in bone remodelling is break down of a matrix, where the major constituent is crystals of hydroxyapatite. The only cell that can resorb this matrix is the osteoclast. It binds to the surface and develops into a highly polarised multi-nuclear cell, where a central part at the bone interface is sealed off by the cell membrane at the periphery of the cell, the so-called clear zone, which mediates attachment. The central ruffled border region mediates bone resorption. It is the location of proton pumps which produce an acid environment in the underlying bone, thereby promoting dissolution of the mineral crystals. The osteoclast, when sufficient resorption of the bone has been accomplished and a resorption pit has developed, detaches and moves to a new site. The defect created becomes repopulated with osteoblasts that produce new bone.

Key issues in this remodelling are the questions of what governs attachment and detachment of osteoclast, and how the polarisation of the cells is induced. What is the background for osteoblast binding to the new surface created in the pit, while it apparently does not compete with the osteoclasts binding to the bone surface before resorption?

To understand the mechanisms involved, we need to identify molecular mechanisms. Therefore, we have studied potential roles of two bone proteins in osteoclast activity. One of these is osteopontin, which represents a major anionic bone protein. The protein backbone of Mr 32,600 is extensively substituted with primarily O-glycosidically-linked oligosaccharides and phosphate groups linked to serine residues. The protein itself contains two potentially functional domains, i.e. a polyaspartic acid sequence with potential for inhibiting hydroxyapatite crystal growth, and that may also mediate the binding of the protein to hydroxyapatite. Another functional domain is the cell binding RGD-sequence.

Osteopontin has been shown to bind osteoclasts in vitro via an $\alpha V\beta 3$ integrin receptor and the interaction can be blocked by synthetic peptides containing the RGD-sequence. This interaction is biologically relevant since we could also show, by using quantitative electron microscopy, that one predominant location of the protein in the bone is at the site of the osteoclast attachment to the mineralised bone surface, while the protein is not found enriched at other sites around the cell, and also not around osteoblasts. In support of the role of the protein as a primary attachment factor, neither of the cell binding proteins, fibronectin and BSP, is enriched at this location. Also, only the $\alpha V\beta 3$ integrin known to bind osteopontin was found at the corresponding location along the cell membrane and only there. Data corroborating the function of osteopontin as the attachment factor for osteoclasts is the observation that RGD-containing synthetic peptides as well as echistatin are potent inhibitors of osteoclastic bone resorption. Interestingly, further studies showed that osteopontin becomes dephosphorylated by the action of TRAP, the osteoclast enzyme shown to be secreted to the environment of the resorption area under the osteoclast. This modification of the protein may serve a role in the detachment of the osteoclasts, since we could show that the dephosphorylated osteopontin no longer mediates binding of the protein in the in vitro system used for testing.

In preliminary experiments relating to osteoblasts repopulating the defect created by osteoclasts, it appears that osteopontin does not support binding of these cells, while BSP supports binding as well as does fibronectin. It is possible then that when bone surface is originally exposed it is covered by osteopontin produced by neighbouring osteoblasts, and serving to recruit only osteoclasts. When this surface has been removed by the activity of these cells, a new surface exposing BSP and/or fibronectin serves to mediate and recruit osteoblasts to fill the defect. Thus, the data indicates an important role for osteopontin in osteoclast-mediated resorption of mineralised bone, which may represent a starting point for developing drugs blocking bone resorption and activating bone formation, respectively.

Gaucher disease, a paradigm for single gene defects

E. Beutler

The Scripps Research Institute, 10666N. Torrey Pines Road, La Jolla (California 92037, USA), Fax + 1(619)5546927

Abstract

Gaucher disease is the most common glycolipid storage disease. Type I, the most common form of the disease, is characterised by enlargement of the liver, and spleen and bone lesions. In the rare type II and type III forms of the disorder, central nervous system involvement is present as well. The disease results from a deficiency of the lysosomal enzyme