List of contributions

The roles of collagen genes in skeletal development and morphogenesis

B. R. Olsen

Department of Cell Biology, Harvard Medical School, Boston, (Massachussetts 02115, USA), Fax +1(617)4320638

Abstract

Molecular genetic analyses of osteochondrodysplasias in mice and humans have recently led to the identification of mutations in genes encoding structural proteins[1] growth factor receptors[2,3] and sulphate transporters[4]. Further analyses of such inherited disorders, using rapid techniques for gene mapping, positional cloning and mutation detection, will undoubtedly uncover other genes that are important for skeletal development. Together with studies of transgenic mice, in which specific genes that are expressed in the skeleton are mutated, these analyses will provide insight into genes that are essential for skeletal morphogenesis.

A good example of the usefulness of the molecular genetics approach for studies of skeletal morphogenesis, is the identification of the mutation causing autosomal recessive chondrodysplasia (*cho*) in mice[5]. Homozygous *cho/cho* mice have severe defects in the cartilage of limbs, ribs, mandible and trachea[6]. They are born with cleft palate, shortened snouts, protruding tongues, short limbs, and die at birth, probably because of asphyxia as a result of tracheal collapse and/or lung hypoplasia[7]. Previous studies have shown that epiphyseal growth plates in *cho/cho* mutants do not show the normal columnar arrangement of chondrocytes as they go through maturation towards hypertrophy and contain fewer hypertrophic chondrocytes than normal growth plate cartilage[8]. Finally, *cho/cho* cartilage is mechanically abnormal in that it is completely without cohesive strength, and the proteoglycan aggregates are unusually extractable. Thus, the *cho* mutation results in abnormalities both in cellular differentiation, cellular organisation and the mechanical properties of the extracellular matrix.

Since several attempts during the past 20 years to identify that *cho* gene were unsuccessful, we decided to determine the chromosomal locus of the mutation by linkage analysis. The results of such analysis showed the *cho* locus to be on mouse chromosome 3, close to the Amy1, Amy2 locus[5]. The gene encoding the α1(XI) chain of the quantitatively minor fibrillar collagen XI was localised to the same region, thus making *Col11a1* a candidate gene for *cho*.

To test the possibility that the *cho* abnormality could be the result of a mutation in *Col11a1*, polyclonal antibodies against a peptide epitope within α1(XI) collagen were used to examine extracts of wild-type and *cho-cho* cartilage by Western-blotting and for immuno-histochemistry. Wild-type extracts showed, as expected, the presence of α1(XI) collagen while no reaction was seen with *cho/cho* extracts, and wild-type cartilage was positive with the anti-α1(XI) antibodies while *cho-cho* cartilage was not. We concluded therefore that the *cho* mutation leads to loss of α1(XI) collagen in cartilage.

Amplification of α1(XI) cDNA by RT-PCR from normal and *cho/cho* RNA, followed by nucleotide sequenc-

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.

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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