Type II Collagen, Proteoglycans and Cartilage Calcification

by

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The calcification of cartilage in endochondral bone formation ordinarily takes place within the growth plate as part of skeletal growth. It is characterised by the assembly of a cartilage matrix by chondrocytes of the proliferative zone. This is later calcified in the hypertrophic zone. The calcified cartilage then largely survives the erosive ingrowth of capillaries which destroy primarily the uncalcified cartilage, such as is found in the transverse septa of primary growth plates. These surviving calcified cartilaginous trabecula then act as a scaffold on which osteoblasts elaborate woven bone, later to be resorbed and replaced with mature bone.

We have been studying the events that occur within the growth plate that lead to and are associated with cartilage calcification. Using sequential transverse microsectioning (20µ thick) of the primary fetal bovine proximal tibial growth plate starting at the transverse fracture face between the last hypertrophic cell and the metaphysis it has proved possible to analyze the chemical changes involving growth plate maturation and calcification. Gel chromatographic analyses of the large proteoglycans that aggregate with hyaluronic acid has revealed that there is no recognizable reduction in size of these molecules in the hypertrophic zone nor in their ability to aggregate. This is contrary to earlier reports. There is, however, a net loss of proteoglycan in the hypertrophic zone (per DNA) as cells enlarged in this zone and matrix volume decreases. The net loss of collagen measured as hydroxyproline is much greater. Type II collagen cleavage can be observed immunohistochemically and it is primarily restricted to the hypertrophic zone. At this time of type II collagen cleavage, type X collagen appears in the upper hypertrophic zone.

Later, the cartilage calcifies in discrete focal sites between collagen fibrils in the lower hypertrophic zone. It mainly occurs not in matrix vesicles but in sites where proteoglycans become concentrated. At this stage, there is no specific association of type X collagen with the focal sites of calcification nor with matrix vesicles which can often be seen
adjacent to these sites. Instead, type X collagen is closely associated with type II collagen fibrils which do not initially calcify in cartilage (unlike in osteoid). Concentrated in these calcifying sites is the C-propeptide of type II collagen which, prior to calcification, is distributed in discrete focal domains in association with type II fibrils. There appears to be a rearrangement of the distribution of the C-propeptide on calcification. The C-propeptide is selectively retained in the matrix compared with collagen and proteoglycan. Within the hypertrophic cells we have observed an apparent accumulation of type II procollagen (bearing the C-propeptide) within the rough endoplasmic reticulum at a time when synthesis of this molecule has been arrested. Type X collagen also displays an intracellular accumulation in these cells but it is not yet known where this occurs. Net procollagen synthesis within the growth plate is maximally increased in growth plate cultures by the addition of 24,25-(OH)_2 vitamin D_3 together with 1,25-(OH)_2D_3. In rachitic rats the former metabolite maximally stimulates synthesis and calcification. Growth plate chondrocytes can be isolated from cartilage matrix using collagenase. These isolated cells can be separated using Percoll gradients. Cells of lowest density contain maximal alkaline phosphatase (which is maximal in the hypertrophic zone) and on culture calcify their newly synthesized matrix. Little or no calcification is observed in cultures of cells of higher density. By studying isolated cells we may be able to conduct further experiment to understand better the interactions of these molecules in calcification.

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