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Novel Events in Cartilage Remodeling During Endochondral Bone Formation

Soha A Soliman

South Valley University, Department of Histology, Faculty of Veterinary Medicine, Qena 83523, Egypt

Abstract: ***Cartilage remodeling is one of the main events occurred to permit invasion of osteogenic cells and deposition of endochondral bone. Many types of cells secrete varieties of proteolytic enzymes such as matrix metalloproteinases (MMP) family and ADAMS (Disintegrin and metalloprotease with thrombospondin type 1 motifs) which exert degradation of cartilage matrix. The current study aimed to specify types of cells involved in cartilage remodeling and distinguish new dramatic events accompanied cartilage remodeling. Bone samples were taken from tibia of quail (Coturnix coturnix) at different ages and processed for light and electron microscopic examination. Two types of chondrocytes; light and dark chondrocytes involved in degradation of the pericellular matrix. Cartilage eroding cells originated form the*** **periosteum***. In the eroding surface, populations of mononucleated and multinucleated cells can be distinguished connected by cytoplasmic processes. Eroding cells were categorized according to heterogeneity. Chondroclasts were characterized by high electron cytoplasmic density, rich in secretory vesicles and covered by microvilli. Chondroclasts acquired polarized profile in lower metaphysis. Two types of heterogenic different osteoblasts were observed; osteoblasts of moderate electron density presented in the tranphyseal cartilage canals and were specialized to deposit the osteoid tissue on the surface of cartilage matrix. This type was not involved in remodeling process. The other type was Intralacunar osteoblasts (light osteoblasts) which possessed the low electron cytoplasmic density, vesicular nucleus and rough endoplasmic reticulum. Light osteoblasts removed the septal matrix of the cartilage in order to get access into the empty lacunae and deposit bone tissue inside the lacunae.***

Keywords: Osteoblasts, Chondroclasts, Remodeling, Endochondral ossification, Bone growth

1. Introduction

Development of skeletal elements is proceeded by two distinctive modes; intramembranous and endochondral ossification. Intramembranous ossification develops the craniofacial bone by direct ossification of embryonic connective tissue membranes. In contrast to endochondral ossification commit longitudinal growth of most of the appendicular skeleton by formation of growing cartilage model. Gradual conversion of cartilage model to bone tissue requires remodeling of extracellular matrix components of the growing cartilage. Matrix metalloproteinases (MMP) are family of proteolytic *enzymes that* responsible for degradation of extracellular matrix proteins and assets in development of *neovasculization* and osteogenic cells migration. Defective remodeling during endochondral ossification implicated in many *skeletal* disorders such as *human* hereditary *chondrodysplasias* ***(1)*** and avian tibial dyschondroplasia **(2, 3).** Many types of cells have been described in degradation process during endochondral ossification; chondroclasts, osteoclasts, monocytes, perivascular cells **(4-7).** Although many investigations documented the *proteolytic* activities of osteoblasts, their role in remodeling during endochondral ossification is obscure. The current study illustrated types of cells specialized in cartilage remodeling and the extent of degradation process of each type and describe new events occurred during endochondral ossification in tibia of quail Coturnix coturnix.

2. Material and Methods

2.1 Collection of Samples

Specimens were collected from the growing bone of apparently healthy quail (Coturnix coturnix) reached 1day,7 days, 2 and 4 weeks of age obtained from the commercial farm of faculty of veterinary medicine of South Valley University. Tibia was carefully excised, fixed in 10% buffered formalin and 4% glutaraldehyde. The growth cartilage was cut out and decalcified in 10 % EDTA.

2.2 Preparation of Paraffin Embedding Specimens

Formalin fixed specimens were dehydrated, embedded in paraffin wax, sectioned, stained with hematoxylin and eosin and safranin O and examined by light microscope.

2.3 Preparation of Resin Embedding Specimens

Glutaraldehyde fixed specimens were postfixed in 1% osmium tetroxide and embedded in spurr's resin. Semithin sections were undertaken, stained with methylene blue and examined by light microscope. Ultrathin sections were stained with uranyl acetate and Reynold's stain and examined by transmission electron microscope.

3. Results

3.1 Types of cartilage models

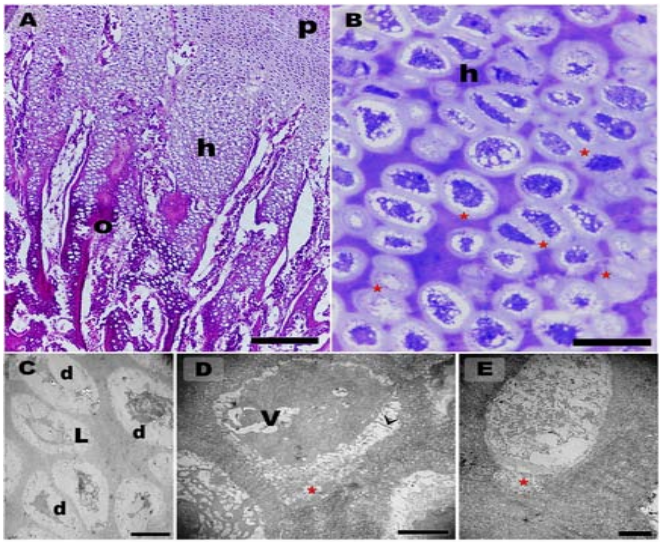
Organization of chondrocytes in the physis of tibia in quail birds contributed in zonal alignment of the growth cartilage into resting, proliferating and hypertrophic zones. Two types of cartilage models were observed. In 1 and 7 days old birds, cartilage model was observed with no endochondral bone formation (fig 2B). After two weeks, the growth cartilage underwent endochondral ossification (fig 2A). The two forms of cartilage models were eroded by the invading osteogenic cells.

3.2 Remodeling of the physeal growth cartilage

Three types of cells were recognized to remodel cartilage matrix; chondrocytes, chondroclasts and light osteoblasts. The purpose and extent of cartilage remodeling of each type of cells were different. Chondroclasts located in the cartilage canals. Population of mononucleated cells of chondroclasts expanded and invaded the cartilage matrix to create the way of the prospective cartilage canals. They preceded the other cells in remodeling process**.** Chondrocytes remodeling was limited to the pericellular matrix aiming to widen the lacunar space and preserving a cartilage scaffold for ossification process. Light osteoblasts remodel the septal matrix to populate the cartilage scaffold and deposited the osteoid matrix on cartilage remnants.

3.2.1 Remodeling by chondrocytes

Hypertrophic zone of the growth cartilage was populated by two types of chondrocytes; light and dark chondrocytes. Dark chondrocytes were defined by the electron dense cytoplasm. Light chondrocytes marked by the electron lucent cytoplasm. Both types were observed to degrade the surrounding matrix. In semithin sections stained by methylene blue, the pericellular matrix has lost their metachromasia (fig 1B). By transmission electron microscopy, the remodeled areas included fragments of the matrix complements (fig 1D, E).



Paraffin section (A) stained by H&E, semi-thin (B) and ultrathin (C-E) sections of the growth cartilage of tibia in 2 weeks (A, B, E) and 4weeks (C, D) old quail. A: Chondrocytes organized into proliferating (P), hypertrophic (H) zones. Note zone of ossification (O). B: Loss of the metachromasia in the pericellular matrix indicating remodeling of the proteoglycan by hypertrophic chondrocytes. C: showed two types of hypertrophic chondrocytes. The electron lucent cells were identified as light chondrocytes and the electron dense cells were the dark chondrocytes. D: Dark chondrocytes. E: Light chondrocytes. Note: The red asterisks refer fragmentation of cartilage matrix which expressed the proteolytic activities of both types of chondrocytes, V: secretory vesicles, arrow head: secretory extrusions. Scale bars represent 80 µm in "A", 20 µm in "B",2 microns in "C", 500 nm in "D, E".

3.2.2 Cartilage eroding cells at the marrow interface

The current study clarify types of cells observed in the transphyseal cartilage canals particularly cartilage eroding cells and the extent of cartilage remodeling of each type. Signs of cartilage remodeling appeared as breaking down of septal matrix (fig 4A, C, fig 5B-F), loss metachromasia of cartilage matrix indicated degradation of proteoglycan in sections stained by methylene blue and fragmentation of cartilage matrix components in ultrathin sections (fig 2B, D). The specimens were examined at the cartilage-marrow interface. Three types of cells were recognized in the transphyseal cartilage canals; chondroclasts, intra-canal osteoblasts and intralacunar (light) osteoblasts. Only chondroclasts (fig 3D, 5B) and intralacunar osteoblasts (4E, 5B-F) were detected to breakdown cartilage matrix. Chondroclasts had electron dense cytoplasm either mononucleated or multinucleated cells connected with each other by cytoplasm processes and located in the transphyseal canals or covered the cartilage surface (fig 3A,F). Chondroclasts was the first observed to undergo the degradation of lower hypertrophic zone. They tunneled through the extracellular matrix in the prospective way of the cartilage canal (fig 3F, G, H). Large population of chondroclasts appeared as syncytial cells spread on both sides of the cartilage matrix along the forming cartilage canals (fig 4D). Chondroclasts exhibited ultrastructural modifications enabling them to remodel the cartilage matrix. Microvilli covering the surface face the degraded matrix and parallel arrays linked by fine threads (fig3 C, D, E). At the site of invasion in lower hypertrophic zone, and mononuclear cells expanded from the giant chondroclasts and projected into the empty lacuna. Multinucleated chondroclasts exhibited unpolarized organization and contained several secretory vesicles (fig 4 A, B). Typical polarized chondroclasts were frequently observed to remodel cartilage matrix in lower metaphysis (fig 4E, F).

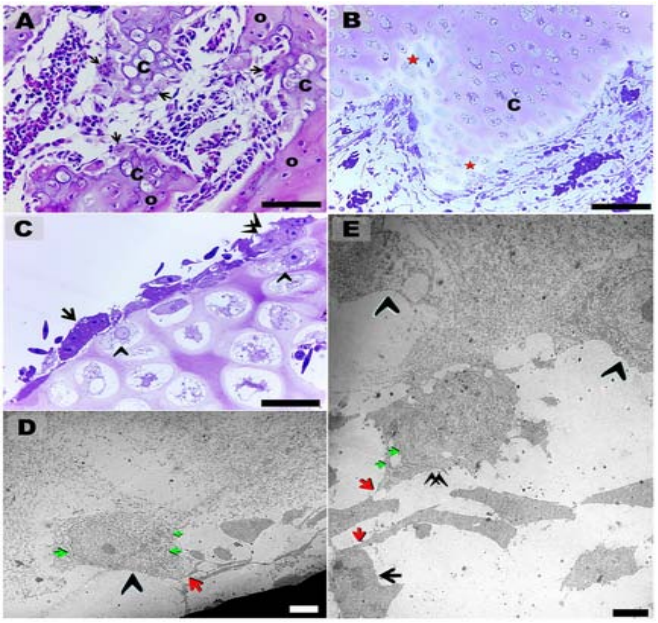


Figure 2: Types of cartilage models during development of skeletal elements of the tibia and cartilage eroding cells at the marrow interface

Paraffin section (A) stained by H&E, semi-thin (B, C) and ultrathin (D,E) sections of the growth cartilage of tibia in 1 day (B, D), 7 days (C, E) and 2 weeks (A) old quail. A: Cartilage model undergo endochondral ossification, Note: The frequency of multinucleated chondroclasts. B: Cartilage model without endochondral bone formation. Note: Loss the matrix metachromasia at the cartilage eroding surface (Red asterisks). C, D, E: Types of cartilage remodeling cells. Chondroclasts (arrows) with high electron dense cytoplasm, light osteoblasts or intra-lacunar osteoblasts contained electron lucent cytoplasm (arrow heads), intra-canal osteoblasts have moderate cytoplasmic density (double arrow heads). Note: The red arrows refer to cytoplasmic processes connected between chondroclasts and light and intra-canal osteoblasts, Green arrows refer to the rough endoplasmic reticulum. Scale bars represent 50 µm in "A, B", 20 µm in "C", 500 nm in "D, E".

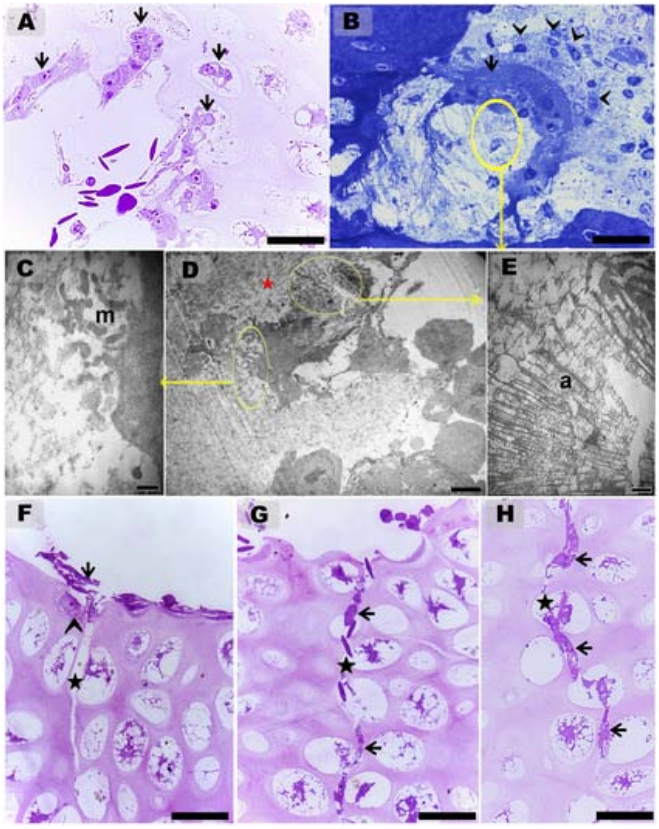


Figure 3: remodeling of the growth cartilage by chondroclasts

Semi-thin (A, B, F-H) and ultrathin (C-E) sections of the growth cartilage of tibia in 1 day (C-H), 7 days (A) and 2 weeks (B) old quail. A: Invading chondroclasts appeared as mononuclear cells. B: Multinucleated chondroclasts Note: The associated light osteoblasts (arrow heads). C, E, F: Surface modification of the invading chondroclasts. Microvilli (m) and array like structure (a). Note: Fragmentation of cartilage matrix (red asterisk). F-H: Chondroclasts preceded the invasion of the other cellular and vascular elements in the prospective way (black asterisks) of cartilage canal. The arrows refer to chondroclasts, the arrow heads refer to the light osteoblasts. Scale bars represent 20 µm in "A, B, F, G, H", 100 nm in "C, E", 500 nm in "D".

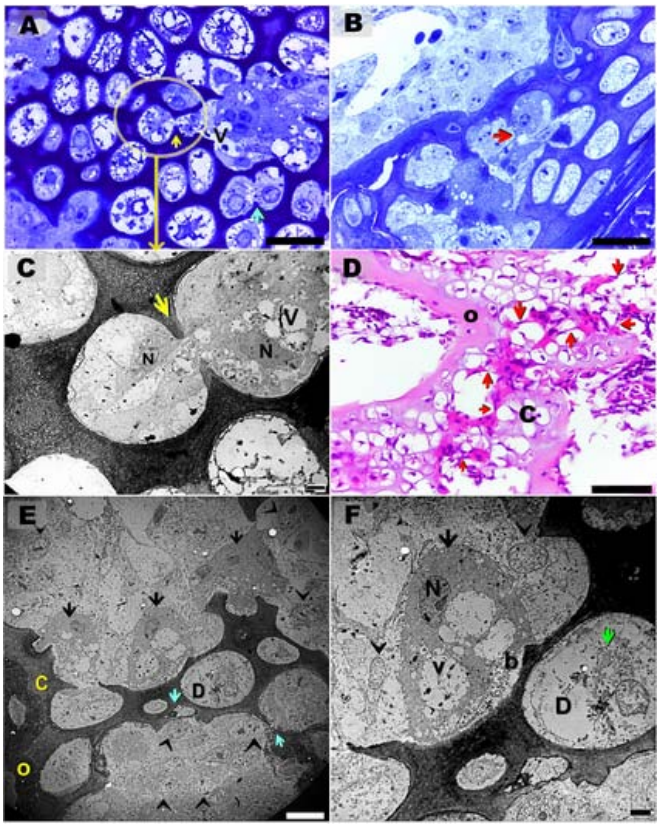


Figure 4: Morphological features of chondroclasts

Paraffin section (D) stained by H&E, semi-thin (A, B) and ultrathin (C, E, F) sections of the growth cartilage of tibia in 2 weeks (D-F) and 4 weeks (A-C) old quail. A, C: un-polarized morphology of chondroclasts. Note mononucleated chondroclasts expanded to the empty lacuna, vesicles (V). B, D: Syncytial population of chondroclastic cells extended along both sides of eroding surface of cartilage canals Note: The red arrows refer to cytoplasmic processes connecting between chondroclasts, cartilage (C), osteoid tissue (O). E, F: polarized morphology of chondroclasts in the low metaphysis. Note: brush border (b), vesicular zone (V), and nucleus (N), cartilage (C), osteoid tissue (O). Dying light chondrocyte (D) was enclosed in a well preserved lacunar matrix, the cytoplasm lost almost of cellular organelles (the green arrow), and arrow heads refer to light osteoblasts. The black arrows refer to chondroclasts, the arrow heads refer to light osteoblasts, Yellow arrows refer to the broken septal matrix and the blue arrows refer to degradation of cartilage matrix by light osteoblasts. Scale bars represent 20 µm in "A, B", 500 nm in "C, F", 50 µm in "D", 2 microns in "E".

Two types of osteoblasts were identified of variant electron densities. Osteoblasts of moderate electron density located in the transphyseal canal and deposited the osteoid tissue on the surface of the cartilage matrix. The intracanal osteoblasts contained RER, mitochondria and secretory vesicles with a central hollow zone (fig 8B, D). The cytoplasmic processes connected between the intra-canal osteoblasts (fig 8B). The second type of osteoblasts had low electron density cytoplasm. This type can be observed in cartilage canals as a multinucleated cellular masses presented in association with the chondroclasts (fig 5A, C, D, 5B, D, F). The low electron dense osteoblasts invaded the empty lacunae and breakdown

only the septa between the lacunae preserving the rest of cartilage matrix. The intralacunar osteoblasts (light osteoblasts) laid down the osteoid tissue to fill the lacunar spaces (fig 6). The margins of cartilage remnants loss the metachromasia indicating osteoblasts continued cartilage proteoglycan remodeling even after started to secrete bone matrix (fig 4E, 7A, C). They contained RER, mitochondria, vesicular nucleus and several vesicles were observed in their cytoplasm (fig 2E, fig 5D, E, F). In the ossification center, areas of osteoid tissue appeared metachromatic enclosing osteocytes (fig 7B) and stained positive for safranin O (fig 7 D).

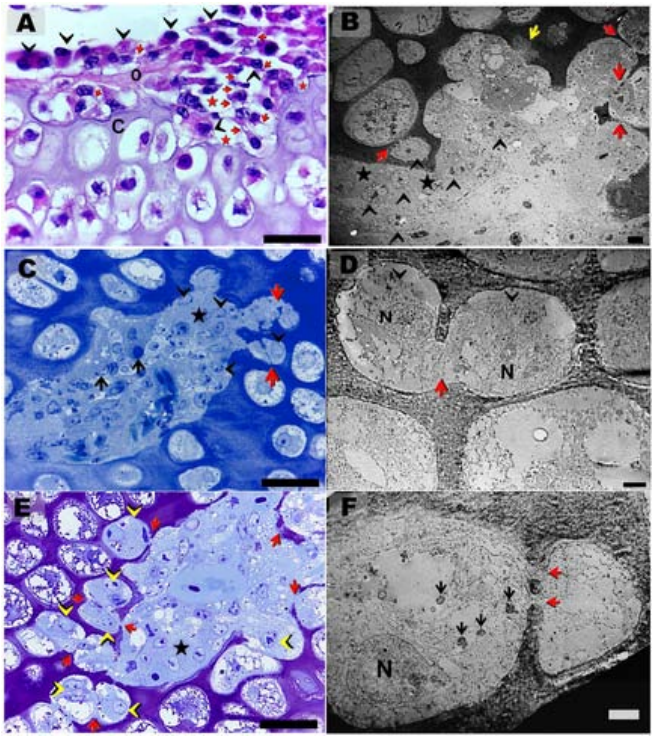


Figure 5: remodeling of the lower hypertrophic zone in growth cartilage by light osteoblasts

Paraffin section (A) stained by H&E, semi-thin (C, E) and ultrathin (B, D, F) sections of the growth cartilage of tibia in 2 weeks (A, C, D, F) and 4 weeks (B, E) old quail. A: osteoblasts deposited osteoid matrix (O) on the surface of cartilage (C). The red asterisks refer to areas of cartilage degradation; red arrows refer to cytoplasmic processes connecting between light osteoblasts, the arrow heads refer to light osteoblasts. B: light osteoblasts (arrow heads) located in cartilage canals in association with chondroclasts (black arrows). Note: Red arrows refer to degradation of septal matrix by light osteoblasts. The yellow arrow refers to degradation of cartilage matrix by chondroclasts. C, D, E: Areas of septal matrix degradation (red arrows), light osteoblasts (black and yellow arrows). Note: The arrows refer to chondroclasts. F: Light osteoblasts breakdown the septal matrix (red arrows), the black arrows refer to phagocytized cartilage matrix. The multinucleated cellular masses of light osteoblasts in the cartilage canals (black asterisks). Scale bars represent 20 µm in "A, C, E",100 nm in "B", 500 nm in "D, F".

Both chondroclasts and osteoblasts were originated from the periosteum. Chondroclast progenitors appeared as syncytial masses of strongly stained cells among pale stained syncytia representing osteoblast progenitors (fig 8C). In cartilage canals chondroclasts and osteoblasts were distinguished and cytoplasmic processes linked between both types of cells (fig 1C, E, fig 8D).

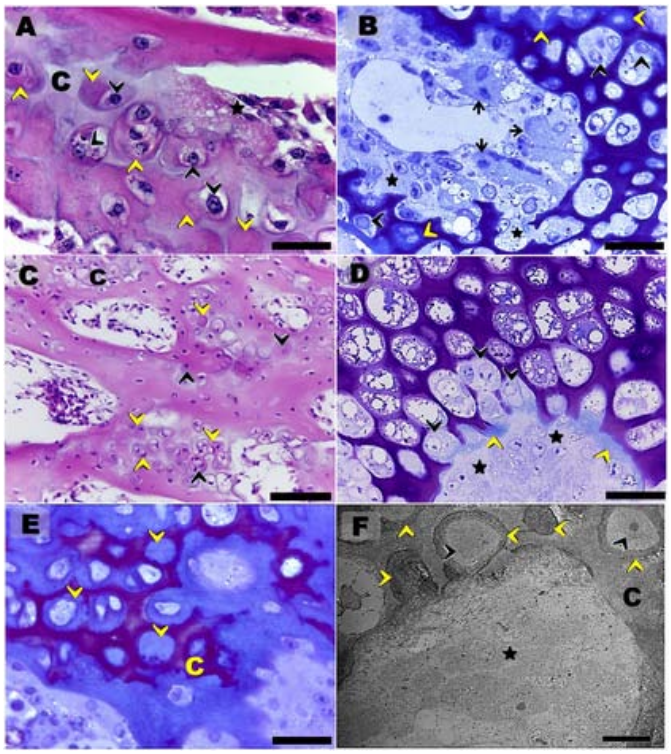


Figure 6: osteoid deposition intra-lacunar by light osteoblasts

Paraffin sections (A, C) stained by H&E, semi-thin (B, D, E) and ultrathin (F) sections of the growth cartilage of tibia in 2 weeks (A-C) and 4 weeks (D-F) old quail. Deposition of bone tissue inside cartilage lacunae (yellow arrow heads) by light osteoblasts (black arrowheads). Note: The multinucleated cellular masses of light osteoblasts in the cartilage canals (black asterisks). Cartilage matrix (C). Scale bars represent 20 µm in "A, B, D, E", 50 µm in "C", 2 microns in "F".

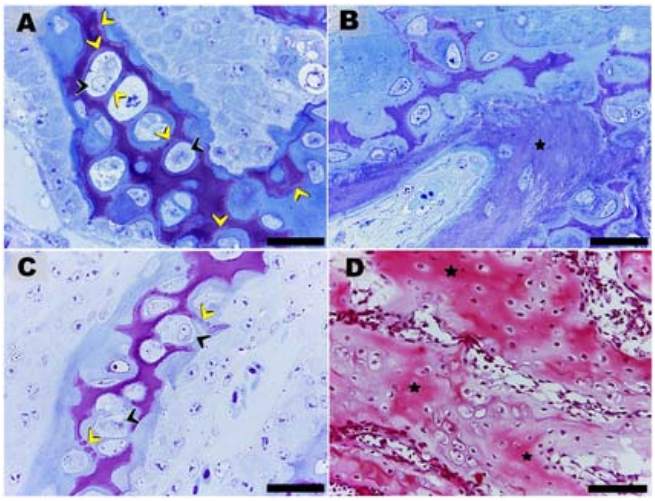


Figure 7: Remodeling of proteoglycan during ossification

Paraffin section (D) stained by Safranin O and semi-thin (A-C) of the growth cartilage of tibia in 2 weeks (D) and 4 weeks (A-C) old quail**.** A: Loss metachromasia (yellow arrows) of the cartilage margins during ossification. B, C: Fragmentation of cartilage matrix (yellow arrows). Metachromatic bone matrix (asterisk) encloses osteoblasts. D: the osteoid tissues enclosing osteocytes stained positive for Safranin O (asterisks). Note: The arrow heads refer to light osteoblasts. Scale bars represent 20 µm in "A-C" and 50 µm in "D".

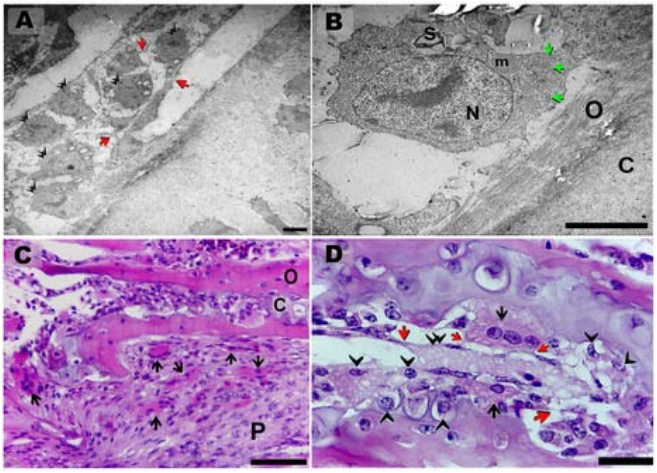


Figure 8: Intra-canal osteoblasts and the syncytial origin of chondroclasts

Paraffin sections (C, D) stained by H&E, semi-thin (A, B) and paraffin (C, D) sections of the growth cartilage of tibia in 2 weeks old quail. A, B: Intra-canal osteoblasts located in cartilage canals and deposited osteoid tissue (O) on the surface of cartilage matrix (C). Intra-canal osteoblasts have moderate electron dense cytoplasm, rough endoplasmic reticulum (green arrows), mitochondria (m), vesicular nucleus (N), and secretory vesicles (S). C: The arrows refer to chondroclasts progenitors emerged from the periosteum (P). D: syncytial cells distinguished to chondroclasts (arrows), multinucleated light osteoblasts (double arrow), individual light osteoblasts (arrow heads) which remodel the cartilage surface. Note: cytoplasmic processes (red arrows) connect between chondroclasts and light osteoblasts. Scale bars represent 500 nm in "A, B", 50 µm in "C" and "20 µm" in "D".

4. Discussion

The current study clarify cartilage remodeling cells, categorize cellular specializations in remodeling process and discuss the developmental events preceding endochondral bone formation. Chondrocytes, chondroclasts and osteoblasts were observed to remodel cartilage matrix in the physeal growth cartilage.

A- Remodeling of cartilage by chondrocytes

Two types of chondrocytes were identified in the hypertrophic zone. Cells contained electron lucent cytoplasm; light chondrocytes and others have electron dense cytoplasm; dark chondrocytes. Light and dark chondrocytes degrade the cartilage matrix. Cartilage remodeling by chondrocytes was not exceed the pericellular matrix and not break the septal matrix. Remodeling activities of chondrocytes were investigated by immunohistochemistry and PCR analysis. Matrix metalloproteinase 13 (MMP-13) is localization in hypertrophic chondrocytes during endochondral ossification of the mandibular condyle and tibiae of newborn mice **(8).** Human adult articular chondrocytes express MMP-2 **(9).** ADAMTS-1 is expressed by adult articular chondrocytes in vivo and in vitro **(10).**

B-Remodeling of the physeal growth cartilage in the marrow front

1- Chondroclasts

Chondroclasts appeared as mononucleated and multinucleated cells of high electron density cytoplasm connected with cytoplasmic processes. Cartilage erosions were detected by both mononucleated and multinucleated cells. Mononuclear cells predominated the primitive form of cartilage model "without endochondral bone formation" in contrast, multinucleated chondroclasts prevailed the other form of cartilage model that undergo endochondral ossification. Similar results are observed in other avian species. In chick embryos, mononuclear acid phosphatase positive cells contained lysosome-like bodies degrade the cartilage matrix **(5).** These cells are described as macrophage **(11).** Histochemical resorbing markers were applied to detect the activities of cartilage resorbing cells. Mononuclear TRAP +ve (Tartrate-resistant acid phosphatase) cells are interpreted as osteoclast or chondroclasts progenitors **(12, 13).**

Multinucleated chondroclasts were numerous in cartilage model that undergo endochondral ossification. Invasive chondroclasts located at the erosion front didn't exhibit polarized morphology. Such cells seem to be analogue to the migrating osteoclasts which tunnel through bone matrix and loss the polarized appearance. In lower areas of the metaphysis, chondroclasts acquired polarized profile. This stage may be identical to the stationary osteoclasts. Similar results were reported in late stages of bone development. cartilage was resorbed by giant cells of chondroclastic type **(5).** large tartrate-resistant acid phosphatase (TRAP) positive multinucleated cells that neither exhibit the typical polarized configuration nor develop ruffled border and clear zone were termed as chondroclasts by **(12, 14, 15)**, inactive osteoclasts by **(13)** or as Preosteoclasts by **(16)** while those acquire polarized organization and locate in Howship's lacunae were commonly known as active osteoclasts **(13, 15).** Some authors describe the polarized cells as chondroclasts **(17, 18).** Typical multinucleated chondro- or osteoclasts profile confeined to the lower level of the subchondral area **(6).**

Chondroclasts were rich in secretory vesicles and may not acquire polarized profile suggesting that establishment of bone-resorbing compartment was none-ssential for remolding and cartilage degradation can depend on production of proteolytic enzymes like other cells have the invasive properties such as trophoblasts during placental formation **(19),** mesenchymal cells during angiogenesis of fetal organs **(20)** and cancer cells during metastasis **(21).**

Osteoclasts are commonly known to originate form hematopoietic tissue. Macrophages have osteoclastogenic potential and can fuse to form multinucleated cells **(22).** Unlike chondroclasts in the current study, results revealed that chondroclasts and osteoblasts emerged from the periostium in cellular syncytium which became distinguish to mononuclear and multinucleated cellular units connected by cytoplasmic processes. This result resembles an old concept developed from 1949 to 1970 that the connective tissue origin of osteoclasts and osteoblasts and both cells are of the same lineage **(23).**

2- Intralacunar osteoblasts (light osteoblasts):

In the present study, light osteoblasts appeared as electron lucent multinucleated cellular cells in cartilage canals. Mononuclear cellular units invaded and remodeled the septal matrix in areas of lower hypertrophic zone and subsequently deposit osteoid tissue. light osteoblasts contained active nucleus, rough endoplasmic reticulum and connected by cytoplasmic processes. In chick embryos, cartilage resorbing mononuclear cells contain euchromatic nucleus with distinctive nucleolus and extensive rough endoplasmic reticulum interconnected with cytoplasmic processes **(11, 16, 17, 24).** In previous investigations, remodeling activities of osteoblasts have been described**.** Osteoblasts exhibit osteolytic activities during transition to osteocytes and acquire the flattened profile **(25).** Osteoblast-like cells produce and secrete active cathepsin B. The production and secretion was stimulated by IL-1β and PTH **(26, 27).** Partially purified cultured human osteoblast produce matrix metalloproteinases (MMPs) collagenase, gelatinase-B and stromelysin in responding to parathyroid hormone (PTH), 1,25-dihydroxy vitamin D3(1,25(OH) D3) **(28).** Collagenase synthesis by cultured mouse osteogenic MC3T3-E1 cells was significantly stimulated by the addition of parathyroid hormone. Characteristic 3/4 and 1/4 fragments of collagen alpha-chain are isolated from the cultured media **(29).** Culture of osteoblast-like cells from a rat sarcoma and osteoblast-enriched populations of rat calvarial cells synthesize and secrete a true collagenase and collagenase inhibitor **(30)**. Gelatinolytic activity is detected in human osteoblasts-conditioned media by SDS-substrate gel electrophoresis **(31).** ADAMTS proteases that target the proteoglycans were investigated in osteoblasts. ADAMTS have identified in Osteoblasts, osteocytes and wide varieties of cells **(32).** Cells of the osteoblast lineage express ADAMTS1, 3, 4 and 5 **(33).** ADAMTS-1 is specifically up-regulated in osteoblasts by the osteotropic agents PTH, PTHrP, and PGE2 **(34).** In vitro, ADAMTS-1, -4 and -5 mRNA are expressed in cultured rat osteoblasts treated with ascorbic acid, beta-glycerophosphate and dexamethasone, molecules. Using immunohistochemistry staining, ADAMTS-1 protein accumulates in osteoblast ECM during differentiation **(35).**

In the ossification center, margins of cartilage remnants loss the metachromasia which implied continual proteoglycan remodeling by light osteoblasts. The newly formed bone may appear metachromatic by methylene blue. Cartilage specific proteoglycans were detected in areas of the osteoid tissue stained positive by safranin O. These results have been explained by previous investigations. Proteoglycan degradation products were described to play a critical role in deposition of organic and non-organic bone constituents. Decorin and biglycan are small leucine-rich proteoglycan (SLRP). Biglycan have been implicated in regulating mineral deposition and crystal morphology, whilst decorin interact with collagen molecules to facilitate assembly of organic matrix. Thus, remodeling of proteoglycan consider as a preparatory stage for ossification process **(36).**

In the present study, osteoblasts exhibited a phagocytic behavior. Small remnants of cartilage matrix were phagocytized by intralacunar osteoblasts. Osteoblasts phagocytic activities have been investigated in vitro and in vivo. In cultured mouse calvariae, Osteoblast-like cells phagocytize collagen fibrils and calcified bone matrix. Phagocytic activities of osteoblasts appear more frequently in the calcifying area **(4).** Osteoblasts engulf apoptotic bodies during alveolar bone formation in the rat maxilla **(37).** Human osteoblasts show a phagocytic response when cultured in presence of foreign particles (titanium (cpTi), Ti-6Al-4V (Ti-A), and cobalt-chrome (CoCr) and ultrahigh molecular weight polyethylene (UHMWPE; GUR 4150) particles) **(38).** Human osteoblast-like cells isolated from periprosthetic osteolytic bone lesion that undergoing hip replacement, phagocytose fine particles of titanium alloy (TiAlV). The alkaline phosphatase positive human osteoblast-like cells acquire macrophage marker CD68 **(39).** Moreover, the ability of osteoblasts to generate acidic environment is reported. Osteoblasts progenitors are described to behave as proton secreting cells **(40).** Human osteoblast-like SaOS-2 cells induce local extracellular acidification responding to parathyroid hormone (PTH) **(41).**

The present study clarified intralacunar osteoblasts or light osteoblasts deposited bone tissue inside the evacuated lacunae in lower hypertrophic zone of the growth cartilage. Osteoblasts markers are detected intra-lacunar adjacent to chondro-osseous junction. Alkaline phosphatase positive mononuclear cells are restricted to the cartilage marrow interface and diffuse to the nearby cartilage matrix and inside the lacunae **(11, 12, 42).** Other investigators observed bone producing cells and osteoid tissue deposit in the cartilage lacunae **(43)**. Mesenchymal cells invade the empty lacunae in which type I collagen deposit **(7).** Based on these observations, some authors thought that the terminally differentiated chondrocytes further differentiate to bone producing cells **(43).**

5. Conclusion

Many types of cartilage remodeling cells were identified in quail during endochondral ossification of tibia. Two populations of chondrocytes were recognized in the growth cartilage; light and dark chondrocytes. Both types remodel the pericellular cartilages matrix in order to widen the lacunae. The morphology of cartilage resorbing cells was variants in different stages of bone development. In early stages of bone development, mononuclear remodeling cells predominated the eroding surface. Two types of cells were

categorized. The chondroclasts cellular units remodeled the cartilage matrix to form the transphyseal cartilage canals which allow osteogenic cells invasion. The second type is osteoblasts. Light or intralacunar Osteoblasts are specialized to remodel the septal matrix in lower hypertrophic zone. In late stages, multinucleated chondroclasts were frequently observed in cartilage canals. Intralacunar osteoblasts act as resorbing cells and shifted their activities to bone producing cells during endochondral ossification. Chondroclasts precursors emerged from the periostium.

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