G Shen
U Hägg
AB Rabie
K Kaluarachchi

Identification of temporal pattern of mandibular condylar growth: a molecular and biochemical experiment

Authors' affiliations:
G. Shen, Department of Orthodontics, School of Stomatology, Shanghai Second Medical University, Shanghai, China
U. Hägg, Orthodontics, Faculty of Dentistry, The University of Hong Kong, Hong Kong SAR, China
A.B. Rabie, Hard Tissue Biology and Repair Research, Faculty of Dentistry, The University of Hong Kong, Hong Kong SAR, China
K. Kaluarachchi, Department of Biochemistry, The University of Hong Kong, Hong Kong SAR, China

Correspondence to:
Dr Gang Shen
Discipline of Orthodontics
Faculty of Dentistry
Sydney Dental Hospital
The University of Sydney
Level 2, 2 Chalmers Street
Surry Hills
NSW 2010
Australia
Tel.: +61 2 9351 8314
Fax: +61 29351 8336
E-mail: gshe6437@mail.usyd.edu.au

Dates:
Accepted 1 March 2005

To cite this article:
Orthod Craniofacial Res **8**, 2005; 114–122
Shen G, Hägg U, Rabie AB, Kaluarachchi K: Identification of temporal pattern of mandibular condylar growth: a molecular and biochemical experiment

Structured Abstract

Authors – Shen G, Hägg U, Rabie AB, Kaluarachchi K

Objectives – Based on the phenomenon that expression of type X collagen and capillary endothelium correlates with endochondral ossification, the prime aim of this study was to establish the temporal pattern of condylar growth in Sprague–Dawley rats by biochemically identifying the expression of these two factors.

Design – Sprague–Dawley rats were divided into five groups representing five different stages during somatic pubertal growth. *In situ* hybridization and immunoperoxidase were performed to examine expression of type X collagen in hypertrophic zone and capillary endothelium in erosive zone of condylar cartilage. Computer-assisted imaging analyses were conducted to allow for a quantitative assessment of the expression of these two factors, from which the temporal pattern of condylar growth was inferred.

Results – (1) Synthesis of type X collagen and emergence of capillary endothelium were critical factors during the transition of condylar cartilage from chondrogenesis into osteogenesis, a biological pathway that leads to endochondral bone formation, the mode through which the condyle grows. (2) Quantitative analyses revealed the temporal pattern of the expression of these two factors, indicating that the thrust of natural growth of the condyle in the rats occurred in concomitance with somatic pubertal growth, featured by an acceleration starting from day 38, a maximum growth rate on day 56, followed by a decrease afterwards.

Conclusion – It is suggested that the biochemical examination of growth markers, such as type X collagen, might be a new approach to accurately depict temporal pattern of condylar growth which is too delicate to be reflected by gross measurement not only in Sprague–Dawley rats but potentially also in other species.

Key words: capillary endothelium; condylar cartilage; growth pattern; type X collagen

Introduction

Mandibular condyle has been regarded as the active growth site for the mandible (1) and therefore becomes the primary focus of functional orthopedic therapy to stimulate mandibular growth (2). It is difficult, however, to elucidate the real effects of orthopedic therapy on condylar growth by deducting the attribution of condylar natural growth from the overall treatment changes, under the circumstance that these elements are always overlapping together. This might be one of the reasons why the skeletal effects of bite jumping therapy still remain disputable (3). A clarification on growth pattern of the condyle is therefore crucial to fully understand mandibular growth changes occurring during the functional appliance therapy.

To define the condylar growth of the humans, currently the most efficient way is to quantitatively relate growth increment, which is obtained by gross measurement of cephalograms, to the passage of time, i.e. the evaluation of temporal pattern of growth. While cephalometric measurement is useful for scrutinizing condylar growth both in size and direction (4), a lot of pitfalls, such as the instability of reference points (5), susceptibility to producing errors (6) and limited consecutive tracings to avoid radiation exposures, compromise its validity and feasibility. Furthermore, the available information drawn from this approach is limited by the lack of longitudinal reference data based on valid measures, where sample size has been small and the age ranges are restricted (7,8). Some studies using cephalometric measurement are open to question when landmark Articulare (Ar) is used for measurement of mandibular length, a parameter commonly used to indicate condylar growth (9). All these factors are accrued to indicate that cephalometric gross measurement might not be able to accurately depict the delicate changes of condylar growth.

Contrary to the human beings, animal experimentations provide an insight into condylar growth by more than just linear measurement. Histological observation has been conducted to monitor condylar growth by identifying cellular response during chondrogenesis of condylar cartilage (10). More recently, biochemical approaches have been adopted to examine the expression of growth factors in condylar cartilage in response to mandibular repositioning, making it possible to reveal intrinsic aspects of condylar growth at molecular or genetic level with animal models (11).

It is widely accepted that the postnatal growth of the condyle is initiated by the transition of cartilage from chondrogenesis into osteogenesis, a biological process termed endochondral ossification (12). During condylar growth, the mesenchymal cells within the articular layer condense and differentiate into prechondroblasts which progressively mature to become hypertrophic chondrocytes (13). In the deepest part of the hypertrophic chondrocytes, the intercellular matrix becomes calcified. The matrix calcification inhibits diffusion of nutrients, ultimately causing the death of the hypertrophic chondrocytes (14). This layer of degenerating cartilage is termed erosive zone, in which transition from chondrogenic to osteogenic pathway takes place. Just beneath the erosive zone, endochondral bone formation then proceeds, causing replacement of degrading cartilage by newly formed bone. The new bone forms over the naked ends of the mineralized cartilage strands, therefore fusing the condylar cartilage to the osseous mass of the ramus and making the mandibular neck extend into a region previously occupied by the condylar cartilage (15). This pointed to an important fact that the increase in condylar size during postnatal growth is fundamentally supported by endochondral ossification, indicating a close association between condylar growth and endochondral bone formation within condylar cartilage.

It has been well established that endochondral ossification of condylar cartilage is regulated and orchestrated by growth factors, the proteins synthesized by chondrocytes (16). Growth factors like transcription factor Sox9 (17), parathyroid hormone-related protein (PTHrP) (18) and matrix metalloproteinase 13 (MMP-13) (19) control condylar bone formation by facilitating and mediating the biomolecular pathway through which chondrogenic phenotype is shifted to osteogenesis. With a serious of animal experimentations and biochemical examinations, Rabie *et al.* have discovered the correlation between synthesis of some growth factors and progression of condylar bone formation (20).

Type X collagen is a member of short-chain collagen family and expressed exclusively in hypertrophic chondrocytes (21). A recent study has reported its role in facilitating and sustaining endochondral ossification of condylar cartilage (22). A strong correlation exists between synthesis of this protein and onset of

endochondral ossification, indicating that this protein is a reliable marker for endochondral bone formation (23). The invasion of capillary endothelium (or neovascularization), on the other hand, is also crucial for the transition from chondrogenesis to osteogenesis in condylar cartilage during natural growth (24). The newly formed vasculature brings bone-making cells into degenerating cartilage and eventually results in new bone formation. Neovascularization has been quantitatively correlated with the endochondral bone formation and is proven to be a good indicator for condylar growth (25).

The prime aim of this study was to identify the temporal pattern of condylar growth by examining expression of growth factor or indicator, namely type X collagen and capillary endothelium, in condylar cartilage during endochondral ossification.

Materials and methods

Animals and tissue preparation

Thirty female Sprague–Dawley rats, 35 days old, were randomly divided into five groups (n = 6), representing different stages of growth (The University of Hong Kong Committee on the Use of Live Animals in Teaching and Research Approval No. 26398). All the rats were kept in a well-controlled temperature-humidity environment. They were fed with normal diet and had a free access to water for 24 h throughout the whole experimental period. The animals of the five groups were, respectively, killed at 38, 42, 49, 56 and 65 days of age. Immediately after the death, the heads were fixed in 10% neutral buffered formalin. After the decalcification, the soft tissue around the temporomandibular joint (TMJ) was removed with care and the ramus was exposed as much as possible. The tissues were then embedded in paraffin in an identical position by adjusting the exposed ramus surface to be parallel to the upper surface of the embedding block. Serial sections of 7 μm were cut through TMJ at sagittal plane using a rotary microtome (Leica RM 2155; Leica Microsystems Imaging Solutions Ltd, Clifton R, Cambridge, UK) and floated onto glass slides.

In situ hybridization for type X collagen mRNA

Preparation of riboprobes

Sense and antisense [α-^{35}S]-UTP riboprobes were generated by *in vitro* transcription from lineralized DNA template using RNA Polymerase T3, T7 and SP6 in transcription buffer with 10 mM DTT, 250 μM ATP, CTP, GTP, 50 units of RNasin, 100 μCi [α-^{35}S]-UTP in a total volume of 20 μl incubated at 37°C for 1 h. The DNA template was removed by adding 40 units of DNase I (RNase free) into the reaction mixture at 37°C for 1 h. Unincorporated [α-^{35}S]-UTP was removed by column chromatography in a Sephadex G50 column. The column was then eluted with aliquots of 200 μl of column buffer. The radioactivity of each tube was determined by liquid scintillation counting (1 μl of each tube was added to 5 ml of scintillation fluid). The RNA probe was precipitated out from the tube with the highest radioactivity by addition of 0.5 volume of 6 M NH_4Ac (pH 5.3) and 2 volume of absolute ethanol. The labeled riboprobe was re-suspended in 0.1 M DTT to give a radioactivity of 2×10^6 cprn/μl followed by addition of 9 volume of hybridization buffer and stored at −20C.

Hybridization of riboprobe

Upon pre-hybridization treatment, the sense and antisense ^{35}S-labeled riboprobe dissolved in hybridization buffer were heated at 80°C for 3 min and then, respectively, applied onto the pretreated sections on the glass slide in minimal amount. The hybridization was carried out in a slide box with the presence of 50% (v/v) deionized formamide and 5× SSC at 50°C for 18 h. The slides treated with sense riboprobe served as negative control. Hybridized sections then were proceeded with post-hybridization washing.

Autoradiography

The hybridization signal was detected by autoradiography by dipping the slide in liquid emulsion K5 (Ilford, Paramus, NJ, USA). The emulsion was allowed to dry and set for 30–45 min. The emulsion-coated slides were put into a slide box, sealed and exposed for 7 days at 4°C before developing. After exposure, the slides were equilibrated to room temperature, and the sections were developed in Ilford Phenisol developer for 2 min followed by stopping with 0.5% (v/v) acetic acid for 1 min and fixing in 30% (w/v) sodium thiosulfate for 6 min. The developed sections were stained with hematoxylin and eosin. Sections were photographed using Kodak Ektachrome EPP 135-36 film on a Zeiss microscope (Zeiss, Axioskop, Oberkochen, Germany) under bright-field illumination with blue filter.

Immunoperoxidase for type X collagen

The primary antibody for type X collagen, provided by Rucklidge *et al.* (26), was raised in rabbits by injection of type X collagen, which was isolated from pepsin-solubilized porcine growth plate cartilage, at 3-week intervals. Antisera were tested on Western blotted collagens and by ELISA against isolated standard collagens. Antisera to type X collagen were found to recognize this collagen in both the native and thermally denatured forms and was suitable for use on immunoblots and on immunocytochemical staining of tissue sections. The antisera raised against type X collagen were found not to cross-react with other collagen types when tested either by immunoblotting (26). The secondary antibodies used was anti-rabbit IgG-peroxidase conjugate preadsorbed with normal fetal bovine serum (Sigma Code No. A-4914, St. Louis, MO, USA).

Immunoperoxidase staining process started with the sections being dewaxed and submerged in 3% H_2O_2 for 10 min. After incubation with hyaluronidase (0.5 U/ml, Sigma Code No. H-6254) and chondroitinase ABC (0.5 U/ml, Sigma Code No. C-2905) for 1 h at 37°C, sections were washed in PBS and then incubated with normal fetal bovine serum (FBS; GibcoBRL Code No. 16000-044, Carlsbad, CA, USA) 1:10 (diluted with 1X TBS) for 30 min. Sections were then incubated overnight at 4°C with the primary antibody. The incubation with secondary antibodies was performed for 1 h at 37°C followed by TBS washing and dips in 3,3-diaminobenzidine (DAB, Sigma Code No. D-5637) for 1–2 min. The sections were then stained with Mayer hematoxylin for 3 min as background staining.

Immunoperoxidase for capillary endothelium

Monoclonal antibody EN 7/44 (anti-human angiogenesis related to endothelial cells) (BMA Code No T-1107, Augst, Switzerland) used in this study positively reacts with budding endothelial cells where the antigen is only found on the apical part of the vessels. Finally, differentiated endothelial cells are not recognized by these antibodies. Secondary antibodies were mouse immunoglobulin (DAKO, Code No. E0354).

Sections were dewaxed and were submerged in 3% H_2O_2 for 10 min. After incubated with protease digestion (Proteinase K, 10 μg/ml, Sigma Code No. P-6556) for 30 min at 37°C, sections were washed in PBS and then incubated with normal rabbit serum (DAKO Code No. X0902) 1:10 (diluted with 1X TBS) for 30 min. Sections were then incubated overnight at 4°C with the primary antibody. The incubation with secondary antibodies was performed for 1 h at 37°C followed by TBS washing and incubation with ABC (DAKO Code No. K0377) 1:100 (diluted with 1X TBS) for 1 h at 37°C. After 1X TBS washing with 0.1% Tween, the sections were dipped in DAB (Sigma Code No. D-5637) for 1–2 min. The sections were then stained with Mayer hematoxylin for 3 min as background staining.

Quantitative evaluation

The quantitative evaluation was conducted via a computer-assisted image analyzing system (Leica Q550IW; Leica Microsystems Imaging Solutions Ltd) with softwares (Leica Qwin Pro., Version 2.2). This system can acquire high-definition digital images from the specimens. Features from acquired images can be automatically selected and recognized by identifying color, shade, shape or texture. This way, the expression of type X collagen was quantified by measuring the area of type X collagen mRNA *in situ* hybridization signals and type X collagen protein immunostaining. Capillary endothelium in erosive zone was also quantified using this system. One-way ANOVA was processed for statistical test with software GraphPad InStat (San Diego, CA, USA).

Results

Biochemical examinations revealed that type X collagen was exclusively expressed in hypertrophic zone (Fig. 1a, b) and capillary endothelium was found in erosive zone of condylar cartilage (Fig. 2). Computer-assisted image analysis quantitatively correlated the amount of expression of these two factors with the passage of time (Table 1), subsequently revealing the temporal patterns of these growth factors. The changes in synthesis of type X collagen and emergence of endothelium in condylar cartilage during the period of pubertal growth were well manifested in the following five different stages.

Day 38

In situ hybridization revealed few signals of type X collagen mRNA within the hypertrophic chondrocytes.

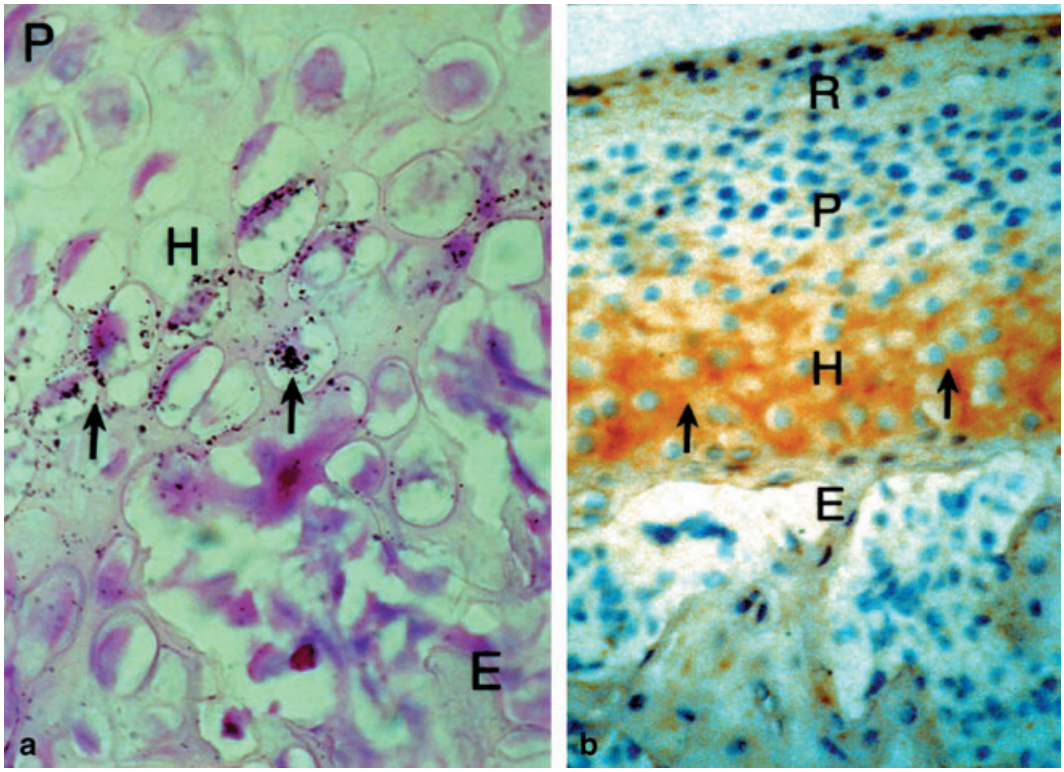

Fig. 1. Biochemical examination of type X collagen in hypertrophic zone of condylar cartilage. (a) *In situ* hybridization signals for type X collagen mRNA (arrows) are expressed within hypertrophic chondrocytes at day 49. (b) Immunoperoxidase for type X collagen protein (arrows) is positive throughout the intercellular matrix of hypertrophic cartilage at day 56 (R, P, H, E are abbreviations, respectively, for resting, proliferative, hypertrophic and erosive zones of condylar cartilage. Original magnification ×300).

The positive immunostaining for type X collagen molecule was not evident. Very little positive immunostaining for capillary endothelium was detected in erosive zone, indicating a weak neovascularization at this stage. Histological observation revealed that the erosive zone was wide and was clearly separated from the hypertrophic chondrocytes above and bony tissue beneath.

Day 42

In situ hybridization showed that type X collagen mRNA signals were localized in hypertrophic zone and were more abundant than those at day 38. Positive immunostaining for type X collagen was detected in the upper region of hypertrophic zone. There was positive immunostaining for endothelial cells in erosive zone, indicating the formation of new blood vessels. Quantitative analysis revealed significant differences between this group and the group of day 38 in amount of type X collagen mRNA signals and the amount of immunostaining both for type X collagen and capillary endothelium (Table 1).

Day 49

In situ hybridization revealed that positive signals of type X collagen mRNA were distributed along hypertrophic zone (Fig. 1a). Quantitative analysis showed that the amount of type X collagen mRNA was at its maximum compared to that at other time points (Table 1). Positive immunostaining for type X collagen was more obvious than that at day 42. The endothelial cells in erosive zone were positively stained with EN 7/44 antibody. Quantitative analysis showed that the amount of type X collagen expression and the amount of immunostaining for endothelium were significantly higher than those at previous stages (Table 1).

Day 56

Quantitative analysis showed that the amount of *in situ* hybridization signals for type X collagen mRNA decreased compared to that at day 49 (Table 1). The amount of immunostaining for type X collagen, however, was at the highest level compared to that in other time points (Table 1, Fig. 1b). Capillary endothelium

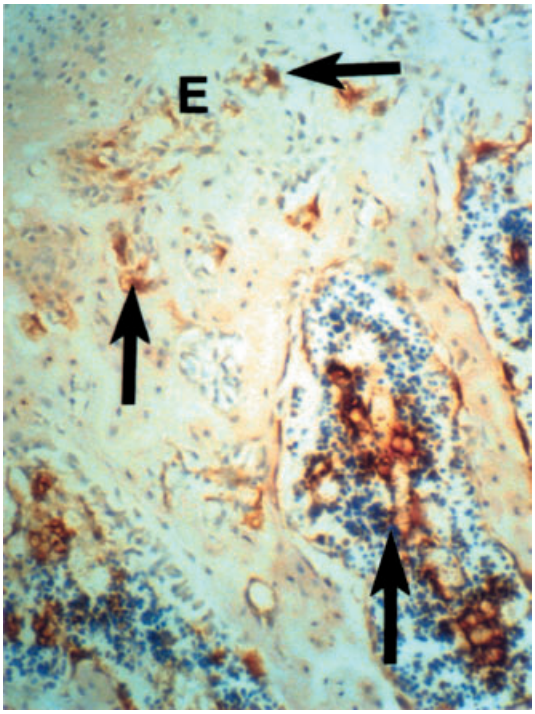

Fig. 2. Immunoperoxidase for capillary endothelium at day 56. Immunoreactions (arrows) are evident in erosive zone of condylar cartilage, indicating enormous invasion of blood vessels during the time of peak rate of condylar growth. Neovascularization in this degenerating area of cartilage brings osteogenic progenic cells and consequently results in new bone formation (E, erosive zone of condylar cartilage. Original magnification ×300).

reached its maximum level evidenced by strong immunoreactions in erosive zone that was close to the underneath endochondral ossification (Table 1, Fig. 2).

Day 65

Few signals for type X collagen mRNA were positively labeled by ^{35}S-labeled riboprobe hybridization. This was further confirmed by quantitative analysis which showed that the amount of type X collagen mRNA signals considerably decreased (Table 1). The amount of immunostaining for type X collagen and for endothelial cells also decreased (Table 1). Histological observation demonstrated that erosive zone became thinner due to the newly formed bone advancing and replacing part of the degenerating cartilage in this zone.

Discussion

In the present study, the expressions of type X collagen and capillary endothelium were examined and quantitatively correlated with endochondral ossification to indicate the temporal pattern of condylar growth.

The amount of type X collagen immunostaining increased between day 38 and day 49 and reached its maximum by day 56, followed by a decrease afterwards (Table 1). This observation was complemented by the temporal pattern of type X collagen mRNA expression, which was similar to that of type X collagen protein (Table 1). However, the maximum expression of type X collagen protein was 7 days later than that of its mRNA which reached the peak level at day 49 (Table 1). This timing discrepancy might result from the biological process involving mRNA transcription and its translation into amino acid sequences to synthesize the protein (27).

It has been believed that type X collagen supports endochondral ossification by regulating the mineralization of hypertrophic matrix (28), and the correlation between type X collagen expression and the progression of endochondral ossification has also been confirmed (29). Rabie *et al.* (30) have found that the temporal pattern of type X collagen expression reflects that of endochondral ossification, the mode through which the condyle grows. Based on the data of the present study regarding the temporal tendency of type X collagen expression, it is reasonable to assume that the velocity of condylar growth in the rat would increase from day 38 and reach the maximum level on

Table 1. **Quantitative analysis of expression of type X collagen and endothelium in condylar cartilage in rats during natural growth (ANOVA)**

Age (days)	38	42	49	56	65
Type X collagen mRNA signals (μm^2)	825 (25)	1297 (55)***	2117 (78)***	1389 (21)***	942 (52)*
Type X collagen immunostaining (μm^2)	6084 (33)	8807 (61)***	10493 (81)***	15470 (121)***	9744 (78)***
Endothelium immunostaining (μm^2)	888 (34)	1886 (42)***	2622 (29)***	3415 (67)***	2092 (71)***

Values represent mean (SD). *$p < 0.05$, ***$p < 0.001$, compared to data at day 38.

day 56, followed by a decrease afterwards. The temporal tendency of condylar growth was also well manifested by cellular response in erosive zone of condylar cartilage where chondrogenesis terminates and osteogenesis begins. Histological structure of erosive cartilage maintained stable between day 38 and day 49. Cartilage degeneration, however, became evident on day 56, indicated by emergence of chondroclasts. At day 65, cartilage in erosive zone continued to break down and its boundary with bony tissue became less distinctive due to the endochondral ossification occurring beneath this particular zone.

The temporal tendency of type X collagen expression revealed in this study is echoed by a study (31) in which the immunohistochemical analysis was conducted to examine type X collagen expression in growth plate of juvenile human being. With advancing age, type X collagen was found to be increasing which paralleled with the light microscopic appearance of 'degeneration' of the epiphysis (31). It has been proposed that type X collagen acts as a calcium-binding protein, thus facilitating the calcification of the hypertrophic cartilage matrix (32). In light of our present findings it is safe to contend that the increased type X expression during day 38 to day 56 might be a biomolecular prerequisite for an increased endochondral bone formation in the condyle.

Urist (33) and Tacchetti *et al.* (34) have found that proliferation and differentiation of mesenchyme cells precede cytodifferentiation and synthesis of cartilage-specific extracellular matrix protein including type X collagen. This points to the linkage between differentiation of progenitor cells and expression of type X collagen. Contrast to epiphyseal growth of long bone which takes place through cleavage of previously differentiated mature cartilage cells, condylar cartilage is covered by articular layer consisting of a thin layer of undifferentiated cells (35). During natural growth, the mitosis of undifferentiated cells results in the migration of the mesenchymal cells out of the covering membrane in the direction of the interior of the condyle (36), then a differentiation takes place in which the mesenchymal cell becomes an immature cartilage cell. Therefore, the new members of the condylar cartilage family have been added through mitosis of undifferentiated mesenchymal cells (15). The premature chondrocytes will progressively mature into hypertrophic phenotype, under which situation type X collagen is synthesized to generate the calcification of the degraded cartilage, a preliminary stage of endochondral ossification (37). This indicates that the expression of type X collagen in hypertrophic zone is linked with proliferation and differentiation of mesenchymal cells upward in the superficial layer of the condylar cartilage. It has been suggested that cellular growth curves show an initial period of rapid progenitor cell proliferation with numbers increasing at an exponential rate (38). Cowan and Morris (39) have proposed that exponential growth lasts as long as the curve of log cell number plot against time becomes linear. However, as soon as the cells differentiate, according to Urist (33), differentiation curtails population size of mesenchymal cells and the consequences are that proliferative activity slows down as development continues. This may explain the increase of type X collagen expression between day 38 and day 56 and decrease after day 56 in the present study.

Neovascularization in erosive zone of condylar cartilage was examined as a supplementary approach to further identify temporal curve of condylar growth. As mentioned before, the invasion of capillary endothelium into the degrading cartilage is a key factor to generate endochondral ossification by brining osteogenic progenitor cells that will eventually lay down bone (40). It is suggested that the temporal pattern of neovascularization reflects that of condylar endochondral ossification and therefore reflects that of the condylar growth. In this study, we identified the temporal pattern of neovascularization in erosive cartilage where transition from endochondrogenesis into osteogenesis takes place. It was found that the temporal pattern of neovascularization resembled that of type X collagen: the amount of positive immunostaining for capillary endothelium increased between day 38 and day 56 and reached the peak level by day 56, followed by a decrease afterwards (Table 1). This indicates that a timing difference might also exist between neovascularization and endochondral ossification, i.e. the occurrence of neovascularization might precede the onset of endochondral ossification. This timing discrepancy is explainable because time is needed for endothelial cells to form the blood vessels which then bring bone-making cells that eventually lead to bone formation.

The lifespan for the laboratory rats is 2.5 years on average. They are sexually mature at 6.5–7.5 weeks of

age and for the female laboratory rats, the first estrous circle comes at 6 weeks of age (41,42). The weight gain records indicate that the somatic growth puberty for Sprague–Dawley rats occurs between 35 and 56 days of age (41). Therefore, the period between day 38 and day 56 that witnessed a rapid growth of the condyle in this study falls in the period of somatic growth puberty. This finding was in agreement with that by Rao and Luo (42), who observed the condylar growth of Sprague–Dawley rats and reported a marked increase during 5–8 weeks of age and an aftermath decease and then a cease.

There have been some studies on temporal pattern of condylar growth for human with normal occlusion using superimposition of cephalograms (43). However, due to lack of the longitudinal reference data, there are different observations and conclusions. Baumrind *et al.* (44) have suggested that condylar growth remains relatively constant between 8.5 and 15.5 years of age. In contrast, Björk (45) has observed condylar growth of 3 mm/year during childhood period, a slight decrease to prepubertal minimum, followed by an adolescent spurt peaking at 5.5 mm/year at about 14.4 years of age. Our finding that the thrust of condylar growth parallels with puberty of somatic growth is in agreement with the findings by Hägg and Attström (46), who have observed greater condylar growth before (11.3 mm/3 years) than after the pubertal peak (9.6 mm/3 years) in a longitudinal follow-up study.

The temporal pattern of condylar growth drawn from biochemical studies with rat experimental model might be inferable to that of human. This is particularly of significance under the situation that the accurate growth curve for human condyle is hard to obtain by approaches of gross measurement, e.g. cephalometric analysis. While the efforts are made to escalate the precision on evaluating human condylar by applying cutting-edge technologies such as computerized 3-D reconstructions (47), the validity of the conclusions from these attempts is still doubtable because inevitable deviations exist between digital simulation and real human tissues.

The concomitance between accelerated condylar growth and the puberty spurt of somatic growth in the rats is also evident in humans, whose increased mandibular growth is concurrent with spurt of overall growth (48). This similarity may indicate a possible existence of the analogy in the temporal pattern of condylar growth between the laboratory rats and humans.

Acknowledgements: This study was supported by The Committee on Research and Conference Grant of The University of Hong Kong and Shanghai Education Research Project. We also thank Dr G. J. Rucklidge of Rowett Research Institute, Scotland for his kind donation of type X collagen antibodies and professional advice on their application.

References

1. Enlow DH. The condyle and facial growth. In: Sarnat BG, Lashin DM, editors. *The Temporomandibular Joint: a Biological Basis for Clinical Practice*. Philadelphia: WB Saunders; 1992. pp. 48–59.
2. Hägg U, Du X, Rabie AB. Initial and late treatment effects of headgear-Herbst appliance with mandibular step-by-step advancement. *Am J Orthod Dentofac Orthop* 2002;**122**:477–85.
3. Bendeus M, Hägg U, Rabie B. Growth and treatment changes in patients treated with a headgear-activator appliance. *Am J Orthod Dentofac Orthop* 2002;**121**:376–84.
4. Pancherz H, Hägg U. Dentofacial orthopedics in relation to somatic maturation. An analysis of 70 consecutive cases treated with the Herbst appliance. *Am J Orthod* 1985;**88**:273–87.
5. You QL, Hägg U. A comparison of three superimposition methods. *Eur J Orthod* 1999;**21**:717–25.
6. Macri V, Athanasiou AE. Sources of error in lateral cephalometry. In: Athanasiou AE, editor. *Orthodontic Cephalometry*. London: Mosby-Wolf; 1995. pp. 125–40.
7. Chen JY, Will LA, Niederman R. Analysis of efficacy of functional appliances on mandibular growth. *Am J Orthod Dentofac Orthop* 2002;**122**:470–6.
8. Buschang PH, Santos-Pinto A. Condylar growth and glenoid fossa displacement during childhood and adolescence. *Am J Orthod Dentofac Orthop* 1998;**113**:437–42.
9. Woodside DG. Do functional appliances have an orthopedic effect? *Am J Orthod Dentofac Orthop* 1998;**113**:11–4.
10. Fuentes MA, Opperman LA, Buschang P, Bellinger LL, Carlson DS, Hinton RJ. Lateral functional shift of the mandible: Part I. Effects on condylar cartilage thickness and proliferation. *Am J Orthod Dentofac Orthop* 2003;**123**:153–9.
11. Rabie AB, Xiong H, Hägg U. Forward mandibular positioning enhances condylar adaptation in adult rats. *Eur J Orthod* 2004;**26**:353–8.
12. Kantomaa T, Ronning O. Growth mechanisms of the mandible. In: Dixon AD, Hoyte DA, Ronning O, editors. *Fundamentals of Craniofacial Growth*. New York: CRC Press; 1997. pp. 189–204.
13. Meikle CM. *Craniofacial Development, Growth and Evolution*. Northfolk: Bateson; 2002.
14. Ross MH, Romrell LJ, Kaye GI. *Histology – A Text and Atlas*. Maryland: Williams and Wilkins; 1995.
15. Garant PR. *Oral Cells and Tissues*. Illinois: Quintessence; 2003.
16. Rabie AB, Hägg U. Factors regulating mandibular condylar growth. *Am J Orthod Dentofac Orthop* 2002;**122**:401–9.
17. Akiyama H, Lyons JP, Mori-Akiyama Y, Yang X, Zhang R, Zhang Z, et al. Interactions between Sox9 and beta-catenin control chondrocyte differentiation. *Genes Dev* 2004;**18**:1072–87.

18. Rabie AB, Tang GH, Xiong H, Hägg U. PTHrP regulates chondrocyte maturation in condylar cartilage. *J Dent Res* 2003;**82**:627–31.
19. Ohkubo K, Shimokawa H, Ogawa T, Suzuki S, Fukada K, Ohya K, et al. Immunohistochemical localization of matrix metalloproteinase 13 (MMP-13) in mouse mandibular condylar cartilage. *J Med Dent Sci* 2003;**50**:203–11.
20. Rabie AB, Tang GH, Hägg U. Cbfa1 couples chondrocytes maturation and endochondral ossification in rat mandibular condylar cartilage. *Arch Oral Biol* 2004;**49**:109–18.
21. Kwan KM, Pang MK, Zhou S, Cowan SK, Kong RY, Pfordte T, et al. Abnormal compartmentalization of cartilage matrix components in mice lacking collagen X: implications for functions. *J Cell Biol* 1997;**136**:459–71.
22. Shen G. The role of type X collagen in facilitating and regulating endochondral ossification of articular cartilage. *Orthod Craniofacial Res* 2005;**8**:11–7.
23. Kong RYC, Kwan KM, Lau ET, Thomas JT, Boot-Handford RP, Grant ME, et al. Intron–exon structure, alternative use of the mouse collagen X gene, colla-1. *Eur J Biochem* 1993;**213**:99–111.
24. Shum L, Rabie AB, Hägg U. Vascular endothelial growth factor expression and bone formation in posterior glenoid fossa during stepwise mandibular advancement. *Am J Orthod Dentofac Orthop* 2004;**125**:185–90.
25. Leung FY, Rabie AB, Hägg U. Neovascularization and bone formation in the condyle during stepwise mandibular advancement. *Eur J Orthod* 2004;**26**:137–41.
26. Rucklidge GJ, Milne G, Robins SP. Collagen type X: a component of the surface of normal human, pig, and rat articular cartilage. *Biochem Biophys Res Commun* 1996;**224**:297–302.
27. Wolfe SL. *An Introduction to Cell and Molecular Biology*. California: Wadsworth Publishing; 1995.
28. Kirsch T, Wuthier RE. Stimulation of calcification of growth plate cartilage matrix vesicles by binding to type II and X collagens. *J Biol Chem* 1994;**269**:11462–9.
29. Nishida T, Kubota S, Nakanishi T, Kuboki T, Yosimichi G, Kondo S, et al. CTGF/Hcs24, a hypertrophic chondrocyte specific gene product, stimulate proliferation and differentiation, but not hypertrophy of cultured articular condrocytes. *J Cell Physiol* 2002;**192**:55–63.
30. Rabie AMB, Shen G, Hägg EU, Kaluarachchi TKPK. Type X collagen – a marker for endochondral ossification of the mandibular condyles. In: Hanada F, editor. *Orthodontics Year Book 2000* (in Japanese). Tokyo: Quintessence; 2000. pp. 50–8.
31. Nerlich AG, Kirsch T, Wiest I, Betz P, von der Mark K. Localization of collagen X in human fetal and juvenile articular cartilage and bone. *Histochemistry* 1992;**98**:275–81.
32. Kirsch T, von der Mark K. Isolation of a human type X collagen and immunolocalization in fetal human cartilage. *Eur J Biochem* 1991;**196**:575–80.
33. Urist MR. Bone morphogenetic proteins. Part 2. In: Rabie AMB, Urist MR, editors. *Bone Formation and Repair*. Amsterdam: Elsevier; 1997. pp. 23–33.
34. Tacchetti C, Tavella S, Dozin B, Quarto R, Robino G, Cancedda R. Cell condensation in chondrogenic differentiation. *Exp Cell Res* 1992;**200**:26–33.
35. Sperber GH. *Craniofacial Development*. Hamilton: BC Decker; 2001.
36. Fawcett DW. *Concise Histology*. New York: Chapman and Hall; 1997.
37. Ogawa T, Shimokawa H, Fukada K, Suzuki S, Shibata S, Ohya K, et al. Localization and inhibitory effect of basic fibroblast growth factor on chondrogenesis in cultured mouse mandibular condyle. *J Bone Miner Metab* 2003;**21**:145–53.
38. Rabie AB, Zhao Z, Shen G, Hägg EU, Robinson W. Osteogenesis in the glenoid fossa in response to mandibular advancement. *Am J Orthod Dentofac Orthop* 2001;**119**:390–400.
39. Cowan R, Morris VB. Cell population dynamics during the differentiation phase of tissue development. *J Theor Biol* 1986;**122**:205–44.
40. Shen G, Rabie B, Hägg U. Neovascularization in the TMJ in response to mandibular protrusion. *Chin J Dent Res* 2003;**6**: 28–38.
41. Lane-Petter W. The laboratory rat. In: UFAW, editor. *The UFAW Hand Book on the Care and Management of Laboratory Animals*. Edinburgh: Churchill Livingstone; 1976. pp. 210–7.
42. Rao Y, Luo SJ. Histological and histochemical observation on the condylar growth and development (in Chinese). *Hua Xi Kou Qiang Yi Xue Za Zhi* 1990;**8**:30–3.
43. Nielsen IL. Maxillary superimposition: a comparison of three methods for cephalometric evaluation of growth and treatment change. *Am J Orthod Dentofac Orthop* 1989;**95**:422–31.
44. Baumrind S, Ben-Bassat Y, Korn EL, Bravo LA, Curry S. Mandibular remodeling measured on cephalograms. 1. Osseus changes relative to superimposition on metallic implants. *Am J Orthod Dentofac Orthop* 1992;**102**:134–42.
45. Björk A. Variations in the growth pattern of the human mandible: longitudinal radiographic study by the implant method. *J Dent Res* 1963;**42**:400–11.
46. Hägg U, Attström K. Mandibular growth estimated by four cephalometric measurements. *Am J Orthod Dentofac Orthop* 1992;**102**:146–52.
47. Nakano H, Watahiki J, Kubota M, Maki K, Shibasaki Y, Hatcher D, et al. Micro X-ray computed tomography analysis for the evaluation of asymmetrical condylar growth in the rat. *Orthod Craniofac Res* 2003;**6**(Suppl. 1):168–72; discussion 179–82.
48. Proffit WR, Ackerman JL. Orthodontic diagnosis: the development of a problem list. In: Proffit WR, Fields HW, editors. *Contemporary Orthodontics*. St Louis, MO: Mosby; 1993. pp. 18–105, 139–86.