Regulatory effects of FGF-2 on the growth of mandibular condyles and femoral heads from newborn rats

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Introduction

The growth regulation of the secondary cartilage of the mandibular condyle is far from completely understood. It was previously suggested that growth of the mandibular condyle is adaptive to functional factors. In the last decades, growth factors have also been shown to be potent regulators of cartilage metabolism. Moreover, it has been suggested that growth factors may differentially regulate the growth of primary and secondary cartilages. However, only a few studies have made a direct comparison of the effects of growth factors on both cartilages. Therefore, the aim here was to compare the effects of FGF-2 on secondary cartilage of the mandibular condyle and primary cartilage of the femoral head from 4-day-old rats in vitro. Cartilages were cultured for 1, 7 and 14 days with 0 and 100 ng/mL FGF-2. We evaluated the effects of FGF-2 on growth, tissue organisation, DNA and glycosaminoglycan (GAG) synthesis and GAG and collagen content. With FGF-2, the morphology of the mandibular condyles changed and the GAG and collagen contents were reduced. However, the growth of the mandibular condyles was not affected. On the contrary, the growth of the femoral heads was strongly reduced due to an inhibition of chondrocyte hypertrophy. In both cartilages, FGF-2 stimulated DNA synthesis in short-term cultures and reduced it in long-term cultures. In conclusion, FGF-2 had a larger effect on the metabolism of the mandibular condyles as compared to the femoral heads. However, the growth of the femoral heads was strongly reduced while that of the mandibular condyles was not affected.

KEYWORDS
Mandibular condyle; Femoral head; Growth; FGF-2; Cartilage

Summary
The secondary cartilage of the mandibular condyle is considered to be adaptive to functional factors. In the last decades, growth factors have also been shown to be potent regulators of cartilage metabolism. Moreover, it has been suggested that growth factors may differentially regulate the growth of primary and secondary cartilages. However, only a few studies have made a direct comparison of the effects of growth factors on both cartilages. Therefore, the aim here was to compare the effects of FGF-2 on secondary cartilage of the mandibular condyle and primary cartilage of the femoral head from 4-day-old rats in vitro. Cartilages were cultured for 1, 7 and 14 days with 0 and 100 ng/mL FGF-2. We evaluated the effects of FGF-2 on growth, tissue organisation, DNA and glycosaminoglycan (GAG) synthesis and GAG and collagen content. With FGF-2, the morphology of the mandibular condyles changed and the GAG and collagen contents were reduced. However, the growth of the mandibular condyles was not affected. On the contrary, the growth of the femoral heads was strongly reduced due to an inhibition of chondrocyte hypertrophy. In both cartilages, FGF-2 stimulated DNA synthesis in short-term cultures and reduced it in long-term cultures. In conclusion, FGF-2 had a larger effect on the metabolism of the mandibular condyles as compared to the femoral heads. However, the growth of the femoral heads was strongly reduced while that of the mandibular condyles was not affected.

Abbreviations: BSA, bovine serum albumin; FGF-2, fibroblast growth factor-2; GAG, glycosaminoglycan; Hyp, hydroxyproline
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factors. Until about 10 years ago, knowledge on the role of growth factors in the regulation of the mandibular condyle was very limited. Much more was already known about their role in primary cartilage.

Primary cartilages are part of the primary cartilaginous skeleton while secondary cartilages appear later in the embryonic development and are independent of the chondroskeleton. They further differ in cellular organization, mode of growth, and composition of the extracellular matrix. Studies on primary cartilage of the growth plate have shown a role of growth factors in the regulation of chondrocyte proliferation, matrix production and terminal differentiation. FGF-2 was therefore suggested to be a chondrocyte mitogen and an angiogenic factor. Insulin-like growth factor-I (IGF-I), fibroblast growth factor-2 (FGF-2), the transforming growth factor-β (TGF-β) superfamily of growth factors, and platelet-derived growth factor (PDGF) are all potent regulators of cartilage growth. IGF-I, TGF-β and FGF-2 have also been detected in the rat mandibular condyle. Furthermore, these growth factors have been shown to affect the metabolism of the mandibular condyle in vitro. FGF-2 was shown to decrease glycosaminoglycan (GAG) and collagen synthesis in the mandibular condyles of neonatal rats, while TGF-β enhanced the GAG and DNA synthesis in mandibular condyles of aged mice. Recently, it was shown that FGF-2 inhibits chondrogenesis in the mandibular condyle in vitro. Differences in the distribution of IGF-I receptors, in IGF-I synthesis, and in the localisation of FGF-2 have been reported between primary cartilages and the secondary cartilage of the mandibular condyle. Therefore, it was suggested that growth factors may differentially regulate the growth of primary and secondary cartilages.

FGF-2 is a potent modulator of proliferation and differentiation in a wide variety of cell types. Also in cartilage, FGF-2 is known to be involved in the regulation of growth and differentiation. In the growth plate, FGF-2 is localised in chondrocytes from the proliferative and upper hypertrophied zones in the chick, the rat and the mouse and also in the hypertrophied and calcifying zones in the chick. FGF-2 was therefore suggested to be a chondrocyte mitogen and an angiogenic factor during endochondral ossification.

Only a few studies describe a direct comparison of the effects of certain growth factors on the primary and secondary cartilages, and indicated that both cartilages are differentially affected. Therefore, the aim of our study was to analyse the regulatory role of FGF-2 on growth and metabolism of the mandibular condyle as compared to the femoral head of newborn rats.

Materials and methods

Cartilages

Cartilages from 55 4-day-old Wistar rats (Harlan, Zeist, The Netherlands) were used. The experiments were approved by the Board for Animal Experiments of the Radboud University Nijmegen. The rats were decapitated and a total of 108 mandibular condyles and 108 proximal femoral heads were dissected under the stereomicroscope. The tissue was continuously immersed in IMDM medium (Life Technologies, Breda, The Netherlands) supplemented with 100 U/mL penicillin and 100 μg/mL streptomycin (Life Technologies). The adhering soft tissue and muscles were removed, and the explants were cut to a length of approximately 1.5 mm. Dissected mandibular condyles contained the cartilaginous tissue of the organ and a small amount of calcified cartilage. Femoral heads contained the proximal epiphysis and a small part of the growth plate.

Tissue culture

The cartilages were placed in a 24-well culture plate (Nunc, Roskilde, Denmark) in 0.5 mL culture medium. The culture medium consisted of IMDM supplemented with penicillin (100 U/mL) and streptomycin (100 μg/mL), ascorbic acid (150 μg/mL) (Sigma, St. Louis, MO, USA), β-glycerophosphate (5 mM) (Sigma) and 0.1% bovine serum albumin (BSA) (Sigma). The culture medium was changed three times per week. The cartilages were cultured at 37 °C in a humidified incubator in an atmosphere of 5% CO₂ in air.

In the first experiment, 54 mandibular condyles and 54 femoral heads were used to determine the effect of recombinant human basic fibroblast growth factor (FGF-2) (Life Technologies) on glycosaminoglycan synthesis and GAG and hydroxyproline (Hyp) contents of the cartilages. Three groups of 18 cartilages (9 mandibular condyles and 9 femoral heads) were cultured with 100 ng/mL FGF-2 and labelled respectively at days 0, 6, and 13, with 10 μCi [³⁵S]-sulphate/mL (Amersham, Little Chalfont, UK) for 17 h, prior to harvesting. Three groups of 18 cartilages (9 mandibular condyles and 9 femoral heads) cultured without growth factor served as control groups. At harvest, six explants of each cartilage type were collected for biochemical analyses and three for histology and autoradiography. The size of the cartilages cultured for 14 days was monitored to determine the effect of FGF-2 on growth.
In the second experiment, 54 mandibular condyles and 54 femoral heads were used to evaluate the effect of FGF-2 on DNA synthesis and GAG and Hyp contents of the cartilages after 1, 7 and 14 days. The second experiment was identical to the first except that the radiolabelled precursor was 5 µCi [methyl-³H]-thymidine/mL (Amersham, Little Chalfont, UK).

Histology and autoradiography

The cartilages were fixed in 4% neutral formaldehyde solution, dehydrated and subsequently embedded in paraffin. Serial sections of 7 µm were cut in the sagittal plane for the condyles and along the long axis for the femoral heads. Central sections were stained with haematoxylin and eosin and the von Kossa method.

To visualise GAG synthesis or cell proliferation, autoradiography was performed on sections of explants labelled either with [³⁵S]-sulphate or with [methyl-³H]-thymidine. In short, sections were coated with Ilford Nuclear Emulsion K5 (Ilford Scientific Product, Mobberley, England), stored at 4 °C, developed, and stained with haematoxylin and eosin.

Growth measurements

In both experiments, repeated standardised pictures were made of the cartilages that were cultured for 14 days, to monitor growth. This was done at the start of the experiment and two times per week. Diapositives were projected on a graphical tablet (Numonics Corporation, Montgomeryville, PA, USA) producing a magnification of 200 times. The outer contours of the cartilages were drawn with a pointer. The calibrated surface areas of the cartilages were calculated using Sigmascan image analysis software (Jandel Scientific, San Rafael, CA, USA). The ex vivo surface area of each cartilage was subtracted from the subsequent measurements to obtain net increase in size per cartilage. This procedure excluded the variation caused by different amounts of non-growing calcified cartilage present in the cartilages.

Biochemical analyses

The synthesis of GAGs was determined from the incorporation of [³⁵S]-sulphate into the tissue while the DNA synthesis was determined from the incorporation of [methyl-³H]-thymidine. After labelling,
the cartilages were washed three times with phosphate-buffered saline (PBS), and digested overnight at 60 °C with 550 μL of 1 mg/mL papain (Merck, Darmstadt, Germany) in 0.2 M NaCl, 0.1 M NaAc, 0.01 M L-cysteine HCl and 0.05 M EDTA, pH 6. Finally, aliquots of the digests and the labelmedia were diluted in scintillation fluid Aqua Luma (Lumac-LSC, Groningen, The Netherlands) and counted in a liquid scintillation counter (LKB-Wallac, Turku, Finland). The rate of synthesis was determined as total incorporation per cartilage to exclude variations due to varying amounts of calcified cartilage in the organs. GAG and DNA synthesis were expressed as percentages of the control (without growth factor) to allow comparison between mandibular condyles and femoral heads.

Furthermore, aliquots of the digests were analysed for total sulphated GAGs with a spectrophotometric assay using dimethylmethylene blue. The Hyp content was determined after alkaline hydrolysis and used as a measure for the amount of collagen. The Hyp and GAG contents were expressed as percentages of the control.

**Statistics**

The data were expressed as means ± standard deviation (S.D.). For the biochemical parameters, differences between the control group and the experimental group and between both types of cartilage were tested by two-way ANOVA. In cases of significant differences, Tukey’s multiple comparisons test was used for further exploration. For the growth analyses, differences in the total increase in size at the end of culture were tested by one-way ANOVA. A P-value of less than 0.05 was considered statistically significant.

**Results**

**Histology**

At day 1, the mandibular condyles of 4-day-old rats showed a thin fibrous layer, a chondroprogenitor and chondroblast layer and a wider layer of differentiated chondrocytes while a broad zone of hyper-

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**Figure 2** Mandibular condyles (A—C) and femoral heads (D—F) of 4-day-old rats (von Kossa staining, bar is 280 μm). At day 1, both cartilages showed some mineralisation (A and D). After 14 days of culture, mineralisation had increased considerably in the control mandibular condyles (B) while in the treated ones it was restricted to the lower part of the organ (C). In the control femoral heads, mineralisation was present in the hypertrophied layer (E) while with FGF-2, some mineralisation occurred in the degenerative zone (F).

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Figure 3  (a) Autoradiography of mandibular condyles of 4-day-old rats (bar is 300 μm). At day 1, \([^{3}H]-thymidine\) incorporation was lower in the controls (A) than in cultures with FGF-2 (B). After 7 days, the labelling intensity of the controls (C) occurred in a reduced zone compared to treated mandibular condyles (D). After 14 days, controls (E) and treated cultures (F) showed a similar intensity. (b) Autoradiography of femoral heads of 4-day-old rats (bar is 300 μm). \([^{3}H]-thymidine\) incorporation at day 1 was observed in all of the non-hypertrophied cartilage and seemed lower in the controls (G) than with FGF-2 (H). After 7 days, the labelling was dispersed throughout the controls (I), whereas its localisation was less uniform with FGF-2 (J). After 14 days, the labelling intensity was higher in the controls (K) than in cultures with FGF-2 (L).
Fig. 3. (Continued).
trophied chondrocytes occupied the lower half of the organ (Fig. 1A). After 14 days in culture, the control mandibular condyles had grown and almost the entire organ was occupied by hypertrophied chondrocytes (Fig. 1B). Only a thin combined fibrous, chondroprogenitor and chondrocyte layer remained. During culture with FGF-2, the fibrous layer had become thicker and a superficial layer containing a lot of small chondroblasts and chondrocytes in few matrix was present (Fig. 1C). The zone of hypertrophy had increased less than in the controls and showed a clear transition to the degenerative zone.

At day 1, the femoral heads of 4-day-old rats consisted mainly of differentiated chondrocytes in an abundant matrix (Fig. 1D). Some chondrocyte hypertrophy was present at the growth plate side of the organ. After 14 days in culture without growth factor the femoral heads had grown, and the hypertrophied layer had greatly increased (Fig. 1E). During culture with FGF-2, the femoral heads had grown less than the controls (Fig. 1F). The zone of hypertrophy was reduced to a few layers before a well-distinguish degenerative zone. The entire organ contained a lot of small chondrocytes.

In the mandibular condyle at day 1, the von Kossa staining indicated some mineralisation in the hypertrophied layer (Fig. 2A). After 14 days, mineralisation had extended locally into the upper part of the control mandibular condyles (Fig. 2B). With FGF-2, mineralisation also occurred but was restricted to the lower part of the organ (Fig. 2C). In the control femoral heads, little mineralisation was found at day 1 (Fig. 2D). After 14 days in culture without growth factor, some mineralisation was observed in the hypertrophied zone (Fig. 2E). With FGF-2, some mineralisation had occurred in the degenerative zone (Fig. 2F).

**Autoradiography**

Autoradiography of mandibular condyles showed that DNA synthesis occurred only in the most superficial layers (Fig. 3a.A–F). At day 1, the labelling intensity was lower in the controls (Fig. 3a.A) than in cartilages cultured with FGF-2 (Fig. 3a.B). After 7 days in control cultures, the labelling was restricted to a thin superficial layer (Fig. 3a.C) while it was still present in the fibrous, prechondroblast and chondroblast layers that formed a much thicker layer in condyles cultured with FGF-2 (Fig. 3a.D). After 14 days in culture, the intensity was lower and quite similar in control and treated mandibular condyles (Fig. 3a.E,F).

In femoral heads at day 1, DNA synthesis was observed in all of the non-hypertrophied cartilage (Fig. 3b.G). With FGF-2, the labelling intensity seemed higher than in the controls (Fig. 3b.H). After 7 days, the localisation of the label had not changed in the control femoral heads (Fig. 3b.I), whereas it was focally concentrated in cultures with FGF-2 (Fig. 3b.J). After 14 days, the labelling intensity was higher in the controls (Fig. 3b.K) than in cartilages cultured with FGF-2 (Fig. 3b.L). In cultures with FGF-2, the label distribution was still less uniform.

**Growth measurements**

The growth of mandibular condyles and femoral heads was measured during 2 weeks of culture with 0 and 100 ng/mL FGF-2 (Fig. 4). The mandibular condyles in the control group showed a total growth of $1.7 \pm 0.5 \text{ mm}^2$ while with FGF-2, the total growth was $1.6 \pm 0.5 \text{ mm}^2$ which was not significantly different (Fig. 4A). In control femoral heads, the total growth was $3.4 \pm 0.4 \text{ mm}^2$ but, with FGF-2, it was only $1.4 \pm 0.3 \text{ mm}^2$ which was significantly lower ($P < 0.05$) (Fig. 4B).
DNA and GAG synthesis

The DNA and GAG synthesis rates of mandibular condyles and femoral heads were analysed after 1, 7, and 14 days in culture with 0 and 100 ng/mL FGF-2 (Fig. 5). At day 1, the incorporation of $[3^H]$-thymidine in the control mandibular condyles was $2.2 \pm 0.1 \times 10^5$ cpm per explant (=100%, control day 1) while that of the control femoral heads was $6.8 \pm 0.9 \times 10^5$ cpm per explant (=100%, control day 1, Fig. 5A). After 14 days, the incorporation in the control mandibular condyles was $3.8 \pm 0.6 \times 10^5$ cpm per explant (=100%, control day 14) and that of the control femoral heads was $8.2 \pm 0.5 \times 10^5$ cpm per explant (=100%, control day 14). With FGF-2, the DNA synthesis of the mandibular condyles significantly increased to 141% of the control at day 1 ($P < 0.05$), while it significantly decreased to 73% of the control at day 14 ($P < 0.05$). In the femoral heads cultured with FGF-2, the DNA synthesis also significantly increased to 138% of the control at day 1, whereas it significantly decreased to 46% of the control at day 14 ($P < 0.05$). At this time, the decrease in DNA synthesis with FGF-2 was significantly stronger in the femoral heads than in the mandibular condyles ($P < 0.05$).

In control cultures at day 1, the incorporation of $[35S]$-sulphate was $1.1 \pm 0.1 \times 10^5$ cpm per mandibular condyle (=100%, day 1) while it was $3.2 \pm 0.5 \times 10^5$ cpm per femoral head (=100%, day 1, Fig. 5B). With FGF-2, GAG synthesis of the mandibular condyles significantly decreased to 75 and 73% of the respective controls at days 1 and 14 ($P < 0.05$). GAG synthesis of the femoral heads was not affected by FGF-2 at any time during culture. A significant difference between mandibular condyles and femoral heads was observed in the FGF-2 groups at days 1, 7, and 14 ($P < 0.05$).

Figure 5 The effects of FGF-2 on DNA synthesis (A) and glycosaminoglycan (GAG) synthesis (B) of mandibular condyles (M) and femoral heads (F) of 4-day-old rats. DNA and GAG synthesis were analysed after 1, 7 and 14 days in culture. Data represent the mean ± S.D. of 6 explants. The results are expressed as percentages of control at days 1, 7 and 14. (*) Denotes a significant difference ($P < 0.05$) between the control and the FGF-2 group. (§) Denotes a significant difference in effect of FGF-2 on mandibular condyles and femoral heads at the same day (two-way ANOVA followed by the Tukey’s test).

Figure 6 The effects of FGF-2 on the hydroxyproline (Hyp) content (A) and the glycosaminoglycan (GAG) content (B) of mandibular condyles (M) and femoral heads (F) of 4-day-old rats. The contents were analysed after 1, 7 and 14 days in culture. Data represent the mean ± S.D. of 12 explants. The results are expressed as percentages of control at days 1, 7 and 14. (*) Denotes a significant difference ($P < 0.05$) between the control and the FGF-2 group. (§) Denotes a significant difference in effect of FGF-2 on mandibular condyles and femoral heads at the same day (two-way ANOVA followed by the Tukey’s test).
Hyp and GAG content

The Hyp and GAG contents of mandibular condyles and femoral heads were determined after 1, 7, and 14 days in culture with 0 and 100 ng/mL FGF-2 (Fig. 6). In control cultures at day 1, the Hyp content of the mandibular condyles was 4.5 ± 1.1 μg/cartilage (=100%, day 1) while that of the femoral heads was 9.5 ± 2.3 μg/cartilage (=100%, day 1, Fig. 6A). At day 14, the Hyp content was 10.8 ± 1.2 μg/cartilage in control mandibular condyles (=100%, day 14) and 21.5 ± 1.5 μg/cartilage in control femoral condyles (=100%, day 14). In cultures with FGF-2, no differences were observed at days 1 and 7. At day 14, the Hyp content of the mandibular condyles had decreased to 60% of the respective control and that of femoral heads to 78% of the control (P < 0.05). At this time, FGF-2 had decreased the Hyp content of the mandibular condyles significantly more than that of the femoral heads (P < 0.05).

In the controls at day 1, the GAG content was 21 ± 2 μg/mmandibular condyle (=100%, day 1) while it was 137 ± 18 μg/femoral head (=100%, day 1, Fig. 6B). No effect of FGF-2 was found at day 1. At days 7 and 14, the GAG content of the control mandibular condyles was respectively 45 ± 4 μg/cartilage (=100%, day 7) and 63 ± 8 μg/cartilage (=100%, day 14). With FGF-2, at days 7 and 14, it was 62 and 55% of the respective control mandibular condyles, which was significantly lower (P < 0.05). In the control femoral heads, the GAG content was 234 ± 15 and 309 ± 28 μg/cartilage, respectively at days 7 and 14. With FGF-2, it significantly decreased to 82 and 81% of the respective controls, at days 7 and 14 (P < 0.05). No difference between both cartilages was found at day 1. However, a significant difference between the two types of cartilage was observed in the FGF-2 groups at days 7 and 14 (P < 0.05).

Discussion

The aim of this study was to analyse the effects of FGF-2 on the growth and metabolism of the mandibular condyle as compared to the femoral head of newborn rats in vitro. Our analyses show that FGF-2 strongly affects the morphology and matrix composition of the mandibular condyle. FGF-2 stimulated cell proliferation, decreased hypertrophy and mineralisation, and reduced GAG and collagen contents. However, growth of the mandibular condyles was not affected. In the femoral heads, the main effect of FGF-2 was a strong reduction of the hypertrophied zone leading to a reduced growth. FGF-2 was previously reported to inhibit chondrocyte terminal differentiation in the growth plate and, recently, also in the mandibular condyle. Our results support the inhibitory effect of FGF-2 on chondrocyte maturation in both primary and secondary cartilages. We also found a reduction of mineralisation by FGF-2. This is consistent with a reduction of alkaline phosphatase activity by FGF-2 found by others. FGF-2, therefore, induced a stronger inhibition of chondrocyte hypertrophy and growth in the femoral head than in the mandibular condyle. However, hypertrophy seems to be more extensive during culture than in vivo. It is therefore possible that the effect of FGF-2 on terminal differentiation has different consequences for the cartilage growth in vivo and in vitro.

We have found differences in the mode of growth of the two types of cartilage, which were also previously described. In the mandibular condyle, appositional cell division was shown in the chondroprogenitor layers and to a lower extent in the chondroblast layers. Interstitial cell division was found in the entire femoral head. For both cartilages, the effects of FGF-2 on DNA synthesis clearly differed between short and long-term cultures. After 1 day in culture, DNA synthesis was stimulated by FGF-2, whereas after 2 weeks DNA synthesis was reduced. Most of the earlier studies were performed on short-term cultures and also reported a mitogenic effect of FGF-2 on both primary and secondary cartilage. However, one study reported an inhibition of proliferation in the perichondrium of embryonic mouse mandibular condyles by FGF-2. In long-term studies, DNA synthesis was also stimulated by FGF-2 in neonatal rat mandibular condyles, and in ovine fetal chondrocytes from the growth plate cultured in serum-containing medium. In our study, certain factors promoting DNA synthesis might be lacking since we used a defined medium without serum to avoid interactions between FGF-2 and serum components.

Differences between short- and long-term cultures were not found for GAG synthesis, which might indicate the existence of a different intra-cellular pathway for its regulation. FGF-2 decreased GAG synthesis in the mandibular condyles, but not in the femoral heads. This corroborates the stronger reduction of GAG content induced by FGF-2 in the mandibular condyles. A decreased GAG synthesis in neonatal rat mandibular condyles by FGF-2 was also found by others. Our data further show that FGF-2 induced a decrease in collagen content which was stronger in mandibular condylar cartilage than in femoral cartilage. Therefore, the matrix composition of mandibular condyles was affected more by FGF-2 than that of the femoral heads. The existence of specific receptors and co-receptors for FGF-2 in
the two types of cartilage might explain some differences. It was reported that the expression of heparan sulfate, a co-receptor for FGF-2, is differentially distributed in mandibular condylar cartilage and in epiphyseal articular cartilage from adult rats. FGF-2 receptors (FGFR2) were found to be distributed throughout the entire femoral head while their expression was more intense in the fibrous and chondroprogenitor layers of the mandibular condyle from newborn rats.

In conclusion, FGF-2 had a stronger effect on the metabolism of the mandibular condyles as compared to the femoral heads. However, the maturation of chondrocytes was impaired more in femoral cartilage. Moreover, FGF-2 strongly reduced growth of the femoral heads while it did not affect that of the mandibular condyles. The regulatory processes of growth and development therefore seem to differ in primary and secondary cartilage, which might be related to their specific origin, structure and function. Interactions between growth factors and specific components of each type of cartilage can modulate their effects and explain certain differences. In addition, the sensitivity of the cells to growth factors and their intracellular pathway may also differ in both cartilages, resulting in a differential control by growth factors. Our results show that the terminal differentiation of primary cartilage in vitro is more sensitive to growth factors such as FGF-2 than that of secondary cartilage. Functional factors may play a more important role in the growth regulation of secondary cartilage, which supports the suggested adaptive role of the mandibular condyle during craniofacial growth.

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References


