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Immunohistochemical PGP 9.5 positivity in human osteoblasts may indicate that compensatory and dysplastic craniofacial growth are under control by peripheral nerves

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Structured Abstract

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Objectives – The purpose was to examine human osteoblasts immunohistochemically in order to clarify the significance of the innervation for alveolar bone growth.

Setting and Sample Population – Unstained sections available from 21 normal human mandibles (foetal gestational ages: 14–22 weeks).

Material and Methods – Before sectioning in 1980–1990, the mandibular tissue had been fixed in 4% neutral-buffered formaldehyde for 5 days. Tissue blocks were then decalcified in equal parts of 2% citric acid and 20% sodium citrate for 7–15 days, dehydrated, embedded in paraffin, and sagittally cut into 4-μm-thick serial sections and mounted on Superfrost® Plus microscope slides. Sections were dried overnight at 40°C. In the present study, paraffin sections were deparaffinized and treated with Tris-EDTA (Merck, Germany), pH 9.0, and immunohistochemically tested with polyclonal rabbit anti-PGP 9.5, and the EnVision™+/HRP dual link (K4065; DAKO Denmark A/S, Denmark) method.

Results – A pronounced protein gene product (PGP) 9.5 activity was registered in osteoblasts from alveolar bone in all specimens. In all cases, the activity was intense at the top of and labially to the alveolar bone, while less or no activity was observed on the inner lingual aspects of the alveolar processes. Osteoclasts and osteocytes reacted vaguely or negatively.

Conclusion – As the present study has demonstrated that human osteoblast activity in the alveolar bone seemingly responds to innervation, it is suggested that the peripheral nervous system via the trigeminal ganglion regulates compensatory and dysplastic alveolar bone formation.

Key words: alveolar bone; craniofacial; growth; immunohistochemistry; peripheral nerve

Introduction

The growth pattern of the jaws during infancy and puberty has been studied in detail by Björk (1, 2), Solow (3, 4) and many others. It has been

demonstrated that growth patterns vary in different craniofacial profiles (2). It has also been documented that growth deviations in one jaw can be compensated by growth activity in the other jaw, the so-called compensatory growth (5). When this compensatory mechanism does not function or even worsens the jaw position and occlusion, the development is considered dysplastic.

Accordingly, in craniofacial growth analysis the terms *compensatory growth* and *dysplastic growth* are commonly used. The mechanisms regulating these growth types are not known. Therefore, it is obvious to focus on factors, which could influence alveolar bone growth in the jaws and furthermore on the ability of these factors to respond on impulses from one jaw to the opposite jaw. It is well known that tooth eruption and alveolar bone growth are interrelated developmental processes and that eruption is important for alveolar bone formation, but a problem in this connection is that the aetiology behind eruption is not known. Parner et al. (6) found that the eruption patterns within fields of the jaws with the same innervation are strongly coordinated, while the eruption patterns in two jaw fields that are innervated by different nerve branches are not coordinated. Therefore, it is obvious to focus on the innervation when studying alveolar bone growth. The innervation to both the upper and lower jaws originates from the trigeminal ganglion, and therefore it is possible to imagine these nerve courses as messengers of growth activity, growth pattern and tooth eruption.

In a study on the early embryological development of the human jaws, it has been shown by immunohistochemical marking of the peripheral nerve course by S100 that the early bone formation of the jaws occurred in close relation to the mandibular nerve, the maxillary nerve, the palatine nerve and the nasopalatine nerve (7). In later studies the early tooth formation and its innervation has been investigated by S100 and by protein gene product (PGP) 9.5, which is a protein expressed in the vertebral neurons and endocrine cells (8). A novel finding in this study on horizontal sections was that osteoblasts were not only positive for S100 but also for PGP 9.5. By nerve growth factor receptor reaction, which also demonstrates peripheral nerves, the early innervation of the tooth bud has been elucidated further (9, 10).

In recent experimental studies it has been shown that cell membranes on osteoblasts have neuroreceptors and therefore affect nerve stimuli (11). In several studies, Lerner and co-workers worked experimentally on nerve receptors on osteoblasts and demonstrated that osteoblast activity depends on the innervation (11). *In situ* hybridization of this interrelationship between nerve tissue and bone tissue has only been vaguely described in literature (12, 13). In studies on human tissue the interrelationship between bone tissue of the jaws and nerve tissue has been studied immunohistochemically in pathological cases such as in osteoid osteoma, which is a benign bone tumour associated with pain (14).

With the new information on neural receptors on osteoblasts it could be hypothesized that human osteoblasts at active growth sites react positively with neuromarkers.

The purpose of the present study was to examine human prenatal osteoblasts, osteoclasts and osteocytes immunohistochemically for PGP 9.5 in sagittal jaw sections. The goal was to clarify whether the innervation could be the key for understanding the compensatory and dysplastic growth responses in the alveolar bone.

Material and methods

Material

Unstained sagittal sections available from 21 normal human mandibles (foetal gestational ages: 14–22 weeks) were investigated. The sectioning was performed in 1980–1990.

Methods

Tissue preparation

Before sectioning, the tissue block from each foetus was fixed in 4% neutral-buffered formaldehyde for 5 days. Tissue blocks were decalcified in equal parts of 2% citric acid and 20% sodium citrate for 7–15 days, dehydrated, embedded in paraffin, and sagittally cut into 4-μm-thick serial sections and mounted on Superfrost® Plus microscope slides (Menzel GmbH & Co., Braunschweig, Germany). Sections were dried overnight at 40°C.

Immunohistochemistry

In the present study, paraffin sections were deparaffinized and treated with Tris-EDTA (Merck, Darmstadt, Germany), pH 9.0, for 2 h at 60°C prior to the

immunohistochemical testing. This was done using the EnVision™ +/HRP dual link (K4065; DAKO, Glostrup, Denmark A/S, Denmark) method as described below. All incubations were performed at room temperature:

Following 2 × 5 min rinses in [0.05 M Tris, 0.15 M NaCl, pH 7.6] Tris Buffered Saline (TBS), endogenous peroxidase was blocked in blocking buffer (S2001; DAKO Denmark A/S). Following a wash in TBS 1 × 5 min, sections were incubated with primary antibody, polyclonal rabbit anti-PGP 9.5, (Z 5116; DAKO Denmark A/S) diluted 1:200 in antibody diluent (S2022; DAKO Denmark A/S). After rinsing 3 × 5 min in TBS, the sections were incubated with peroxidase labelled polymer (K4065; DAKO Denmark A/S) for 30 min. Following washes 3 × 5 min in TBS, sections were incubated in substrate/chromogen 3,3'-diaminobenzidine tetrahydrochloride (DAB) (Sol. 1, K4065; DAKO Denmark A/S) in distilled H_2O for 5 min, and sections were counter-stained with haematoxylin Mayer (LAB00254; Bie & Berntsen, Herlev, Denmark), dehydrated and cover slipped using the pertex mounting media (Histolab, Göteborg, Sweden).

The results were based on the visual comparison of PGP 9.5 immunoreactivity in the tissue sections using a light microscope (Leica, Nussloch, Germany). PGP 9.5-positive cells exhibited a brownish cytoplasmatic colour reaction.

A weak unspecific staining of soft tissue and epithelium was observed, but according to the data sheet this was to be expected.

Results

A pronounced PGP 9.5 positivity was registered in osteoblasts from alveolar bone in all specimens, while osteoclasts and osteocytes reacted vaguely or negatively (Fig. 1). In all cases, osteoblast activity was intense at the top of the alveolar bone and on the external aspects, while less or no osteoblast activity was observed on the inner lingual aspects of the mandible. The findings confirmed that human osteoblasts are PGP positive during active growth and presumably respond to innervation impulses. This finding supports the hypothesis that the innervation could be a factor of importance for understanding the compensatory and dysplastic reactions in postnatal jaw development, illustrated in Fig. 2.

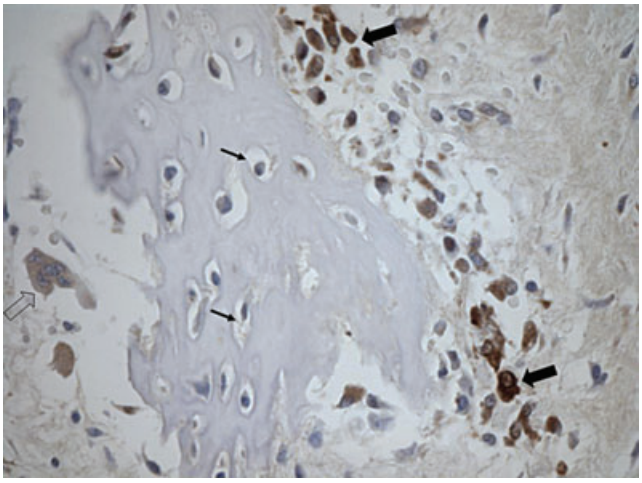

Fig. 1. Histological sagittal section of a mandible from a human foetus, 21 weeks of gestational age. The section illustrates the alveolar bone labial to the right and lingual to the left. The section has been stained immunohistochemically for protein gene product (PGP) 9.5. Positive reaction is shown in brown colour. Intense PGP 9.5 activity, marked by large solid black arrows, is seen in the osteoblasts close to the top of the alveolar bone and labially to the alveolar bone. Vague reaction is also seen in the osteoclasts at the inner lingual aspect of the alveolar bone, marked by open arrow. The osteocytes in the alveolar bone, indicated by small arrows are not positive for PGP 9.5 (×250).

Discussion and conclusion

The present study suggests that osteoblasts may be susceptible to nerve impulses. PGP 9.5 positivity has previously been demonstrated in human pathological osteoblasts (14), while PGP 9.5 positivity in human normal osteoblasts has not previously been described according to location. In the present study the PGP 9.5 reaction indicates differences in growth activity in different jaw regions. A similar approach to jaw growth has not previously been described immunohistochemically. Other neuroendocrine markers such as chromogranin A may supplement the present results.

In the orthodontic clinic, expressions such as *compensatory* and *dysplastic alveolar bone growth* are commonly discussed without knowing how or why these regulatory mechanisms are activated. It would be natural to suppose that compensatory and dysplastic growth of the osseous tissue is regulated by a network of peripheral nerves.

In this connection it is important to focus on three main innervation paths in the maxilla as well as in the mandible (15, 16). These different paths may explain why only parts of the jaws can respond compensatorily or dysplastically during craniofacial development.

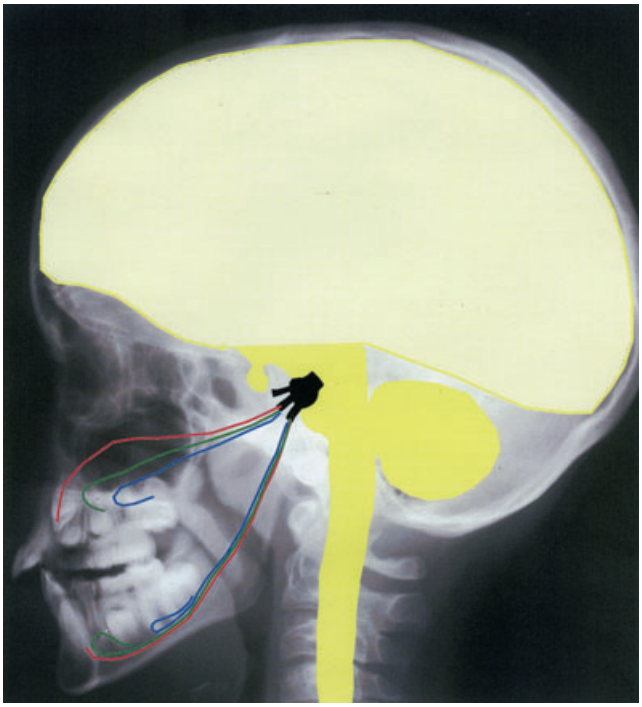

Fig. 2. A profile radiograph of a girl, 9 years of age. Inserted on the radiograph are coloured areas indicating the central nervous system (yellow) and lines indicating the peripheral nervous system to the teeth, alveolar bones and jaws. Black figure indicates the trigeminal ganglion. Red lines indicate innervation to the incisor region, green line to the canine, premolars and blue lines to the permanent molars. It is suggested that the peripheral nervous system via the trigeminal ganglion regulates compensatory and dysplastic alveolar bone formation.

How and why these compensatory and dysplastic impulses develop and function between the jaws is not known. It could possibly be the same process behind referred pain, where pain is felt in another jaw region than the one in which the process causing the pain occurs (17). Also, references between symptoms and pain from the central nervous system, such as headache and tooth pain, have been reported (18).

The problem raised in the present study originates from a clinical radiological observation of osseous growth measured cephalometrically and expressed statistically. The present study suggests that osteoblasts may be susceptible to nerve impulses, but it does not show how these osteoblasts are innervated or how the innervation is stimulated. Thus, the clinical problem raised cannot be solved solely in this immunohistochemical study, but the presence of neuroreceptors on osteoblasts and the intense PGP 9.5 positivity in osteoblasts located in growth areas might indicate that the compensatory and dysplastic developmental processes could be controlled by the innervation.

The histochemical observation, which has been made in this study, is verifiable, but the transfer of the prenatal immunohistochemical findings to the postnatal radiological findings is speculative. A precise verification of the hypothesis involving biopsies from growing children is not possible.

The compensatory and dysplastic growth of the alveolar bone are important for orthodontic diagnostics and treatment planning. The mechanism behind these different growth patterns has never been understood. The present study points towards innervation of the jaws as the factor responsible for the pattern of alveolar bone growth. Previous electromicroscopic studies have proved the existence of myelinated and non-myelinated nerve ends in cortical bone (19, 20).

Whether orthodontic appliances with magnets have the ability to interfere with the peripheral nerve impulses and thus influence the alveolar bone growth might be a question for future studies.

Clinical relevance

Compensatory and dysplastic growth of the alveolar bone are important to be recognized in orthodontic diagnosis and treatment planning. The mechanism regulating these different growth patterns has never been fully understood. The present study points towards innervation of the jaws as the factor that might be responsible for the pattern of alveolar bone growth.

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