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Spatiotemporal expression of NGFR during pre-natal human tooth development

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Structured Abstract

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Objectives – The relation between nerve growth factor receptor (NGFR) in the human pre-natal tooth buds and the dental follicle was investigated. In particular, we sought to determine if there is a specific pattern of p75NGFR expression in developing human tooth buds and their surrounding tissue.

Setting and Sample Population – The Department of Orthodontics at Copenhagen University, Denmark. Histological sections from 11 fetuses, aged 11–21 gestational weeks.

Method – The sections were studied by conventional immunohistochemistry.

Results – Specific spatiotemporal patterns of p75NGFR reactions were observed in the tooth buds and dental follicle: Before matrix production by the ameloblasts, the entire inner enamel epithelium and the entire dental follicle display p75NGFR immunoreactivity; after matrix production is initiated, the immunoreactivity of the matrix producing cells is lost, as is that of the dental follicle adjacent to these matrix-producing cells.

Conclusion – A unique spatiotemporal distribution of NGFR in the pre-eruptive human tooth bud was demonstrated.

Key words: dental follicle; human pre-natal teeth; inner enamel epithelium; nerve growth factor; p75NGFR

Introduction

Nerve growth factor (NGF) is an important neurotrophin (NT) for the development, differentiation, function, and survival of neurons (1). Based on detection of receptors for NGF it has been proposed that NGF also has an effect on many non-neural tissues. Different receptors for NGF have been identified in recent years (2–8). The low-affinity 75-kDa receptor (p75NGFR) (3, 9) is the most abundant.

Nerve growth factor receptor (NGFR) expression has been investigated in relation to the developing tooth bud. In these studies, the locations of NGF and NGFR have been described in primary tooth buds for different developmental stages in rodents (10), and in sections of pre-natal human tooth buds (11).

In the early bud stage human dental development, (gestational week 11), NGFR was observed in the ectomesenchyme. After 1–2 weeks no immunoreactivity for NGFR was observed in the ectomesenchyme, but the inner enamel epithelium was positive for NGFR; the fibroblast cells which later develop into the tooth follicle also showed reactivity. In the cap stage the entire inner enamel epithelium and the cells in the dental follicle were positive for NGFR, but the ectomesenchyme in the tooth papilla was negative (11).

With the initial matrix production by the ameloblasts and odontoblasts, the NGFR expression changed. Those ameloblasts producing enamel matrix were negative for NGFR. The cells in the dental follicle and stratum intermedium were still positive (11). Given this finding, we thought it important to investigate the expression of NGFR in the dental follicle in a more mature period of tooth development, perhaps elucidating the mechanism behind early tooth eruption.

Teeth develop in the alveolar bone, and during crown formation there are small pre-eruptive movements within the jaws also called the pre-eruptive alveolar bone stage. After crown formation is completed and the root begins to form, an active eruption process moves the tooth (12).

Intraosseous tooth eruption is not possible without formation of an eruption path. The fact that bone resorption and bone formation are polarized around erupting teeth and that these events depend upon the adjacent part of the dental follicle strongly indicate that tooth eruption is regulated by the dental follicle (13–15).

The relationship between NGFR expression in the dental follicle and tooth eruption is unknown. We present here the spatiotemporal expression of p75NGFR in the developing tooth buds of 11–21 week human fetuses.

Materials and methods

The samples consisted of formalin-fixed craniofacial tissue from 11 human embryos/fetuses obtained from legally approved therapeutic and spontaneous abortions. Informed consent was obtained according to the guidelines of the Helsinki Declaration II. The fetuses were autopsied at 'Rigshospitalet', University Hospital Copenhagen (before 1990) and at the Hvidovre University Hospital Copenhagen (after 1990) in Denmark. Macroscopic and microscopic examination indicate that all fetuses included in this study were normal, and the overall development was in accordance with gestational age, which was 13–22 weeks.

Histology

The craniofacial tissues from each fetus were decalcified in equal parts of 2% citric acid and 20% sodium citrate [pH 5.6] from 1 to 15 days, dehydrated and then embedded in paraffin. Serial sections were cut sagittally, axially, or coronally at 4 μm thickness. For morphological orientation, every fifth section was survey-stained with Toluidine blue [pH 7.0] and every tenth section was stained with Alcian blue/van Gieson [pH 3.0].

p75NGFR immunohistochemical staining

Paraffin-embedded sections were deparaffinized and rehydrated with 2x xylene, decreasing alcohol and water. Sections were placed in citric acid buffer [10 mM, pH 6.0] in 37°C for 2 h. All incubations were performed at room temperature unless otherwise stated. After rinsing in tap water for a short while, sections were pre-incubated in 4% skimmed milk powder in Tris-buffer [100 mM Tris, 150 mM NaCl, pH 7.6] for 30 min. The tissue sections were then incubated for 60 min with primary monoclonal antibody p75NGFR (M3507, DAKO ChemMate, Copenhagen, Denmark) diluted 1:50 in 2% skimmed milk powder in antibody diluent (S2022, DAKO ChemMate, Copenhagen, Denmark). After rinsing 3×5 min in Tris-buffer, the tissue sections were incubated with biotinylated secondary antimouse antibody (K5055, DAKO ChemMate, Copenhagen, Denmark) for 30 min. The sections were rinsed in Tris-buffer, and reactions were developed in Fast Red (K5055, DAKO ChemMate, Copenhagen, Denmark) for 10–20 min. The reactions were stopped in water, and the tissue sections were then counterstained with Hematoxylin Mayer (LAB00254, Bie & Berntsen, Copenhagen, Denmark) for 30 s. After rinsing in tap water for 5 min, the sections were dehydrated and coverslipped using Pertex® (Histolab, Västra Frölunda, Sweden). The results were based on the visual examination of p75NGFR-immunoreactivity in the tissue sections by light microscopy (Leica, Wetzlar, Germany).

Control immunohistochemical staining

Two types of control reactions were performed. First, the primary antibody was replaced by an isoform of the primary antibody (mouse IgG1 antibody, X0931, DAKO ChemMate, Copenhagen, Denmark). Secondly, the primary antibody was deleted and sections were incubated only with 2% skimmed milk powder in antibody diluent. Control sections were processed in an identical manner as described above for p75NGFR-immunoreactivity. Both types of control sections did not display p75NGFR-immunoreactivity.

Results

Specific developmental patterns in three defined periods were observed.

Period 1 (one fetus)

At 13 gestational weeks (early cap stage), p75NGFR-immunostaining is seen in the developing inner enamel epithelium; the fibroblast cells which constitute the developing dental follicle also show intense immunostaining (Fig. 1).

Period 2 (five fetuses)

At 14–16 gestational weeks (late cap stage), p75NGFR-immunostaining is seen in the entire inner enamel epithelium; the dental follicle, which now completely surrounds the tooth bud, also shows intense immunostaining (Fig. 2).

Period 3 (five fetuses)

At 16–21 gestational weeks (bell stage), p75NGFR-immunostaining is still observed in the inner enamel epithelium and dental follicle, but the ameloblasts which are now producing matrix are no longer immunoreactive and that region of the dental follicle corresponding to the region of matrix-producing ameloblasts also lack immunoreactivity (Fig. 3).

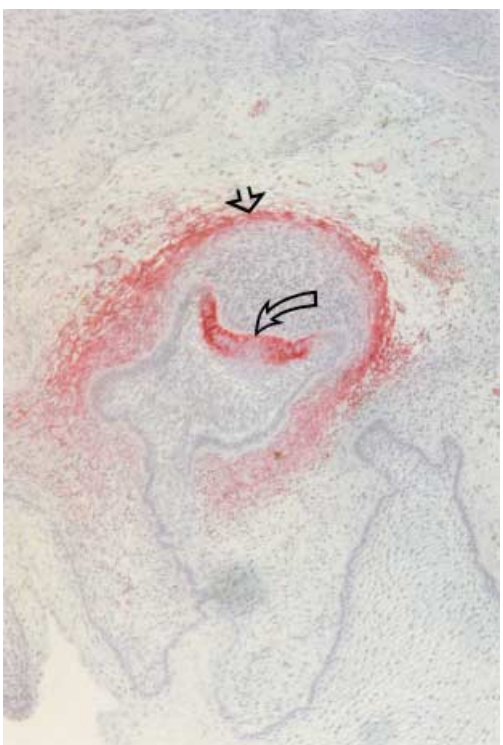

Fig. 1. Early cap stage age: 11–13 gestational weeks. Strong immunostaining is seen in the developing inner enamel epithelium (curved arrow) and the fibroblast cells which constitute the developing dental follicle (straight arrow).

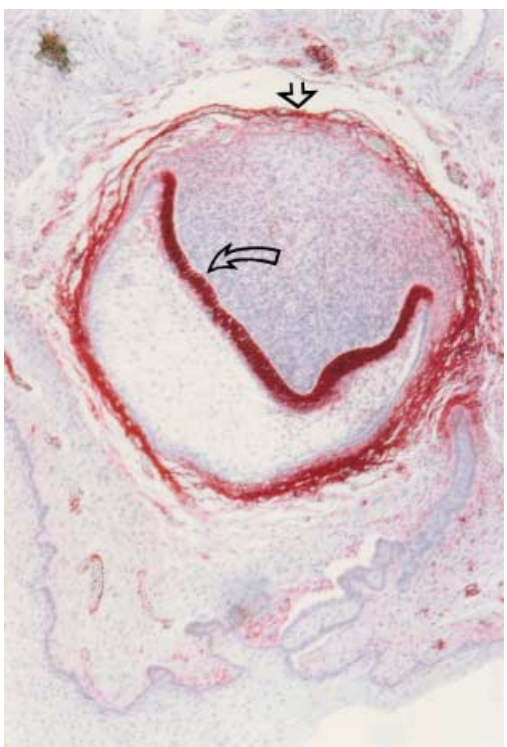

Fig. 2. Late cap stage age: 13–16 gestational weeks. Very intense immunostaining is seen in the entire inner enamel epithelium (curved arrow) and the dental follicle which surrounds the tooth bud (straight arrow).

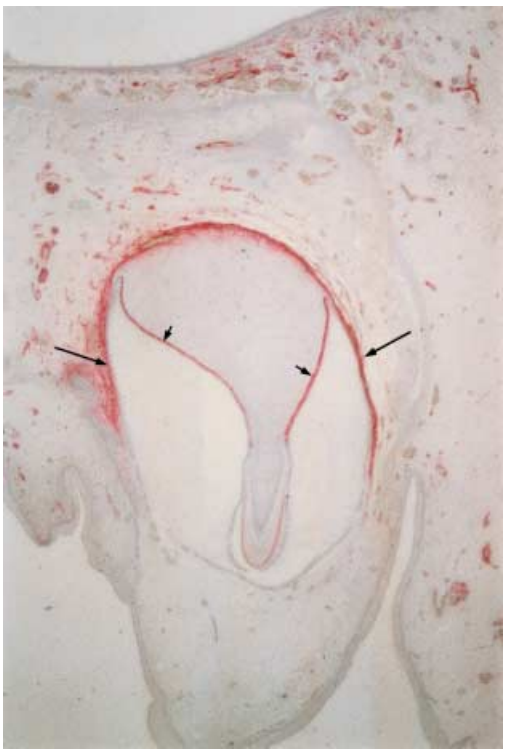

Fig. 3. Bell stage age: 16–21 gestational weeks. Less intense immunostaining is observed in the inner enamel epithelium and dental follicle. When the first matrix from the ameloblasts and odontoblasts is formed, a change in p75NGFR expression is observed in the inner enamel epithelium (small arrows) corresponding region of the dental follicle (large arrows), such that the ameloblasts now producing matrix are no longer immunoreactive, nor is the adjacent dental follicle.

Discussion

The distribution of NGFR in the developing human tooth has been investigated, but the simultaneous appearance and disappearance in the inner enamel epithelium and the dental follicle has not been described.

Many experimental studies have documented the developmental events in the dental follicle and in the enamel organ prior to and during dental eruption.

Analyses of the dental follicle and enamel organ of rat molars have demonstrated that there is an influx of mononuclear cells (monocytes) into the dental follicle just before eruption begins. These monocytes fuse to form osteoclasts that resorb alveolar bone, resulting in an eruption pathway (14, 16).

Colony-stimulating factor-one (CSF-1), transforming growth factor-β (TGF-β), monocyte chemotactic protein-one (MCP-1), Interleukin-1α (IL-1α) and epidermal growth factor (EGF) are some of the growth factor molecules that have been detected in the dental follicle (12, 17–21). It is likely that the presence of these growth factors induce cellular events (monocyte attraction and subsequent osteoclast formation) which are critical to the timing and mechanism of tooth eruption.

Obviously there are no experimental studies investigating the molecular events taking place in human tooth eruption.

However, EGF receptors have been demonstrated in the PDL of erupting human premolars (17).

In the present study we demonstrate the unique spatiotemporal distribution of NGFR in the pre-eruptive human tooth bud (Figs 1–3). Empirical studies such as these, coupled with non-human mammalian studies, will permit us to infer the human eruption mechanism even in the absence of experimental research.

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