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Notch2 protein distribution in human teeth under normal and pathological conditions

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Abstract

Notch signaling is essential for the appropriate differentiation of many cell types during development and, furthermore, is implicated in a variety of human diseases. Previous studies have shown that although the Notch1, -2, and -3 receptors are expressed in developing and injured rodent teeth, Notch2 expression was predominant after a lesion. To pursue the role of the Notch pathway in tooth development and disease, we have analyzed the expression of the Notch2 protein in embryonic and adult wounded human teeth. During the earlier stages of tooth development, the Notch2 protein was expressed in the epithelium, but was absent from proliferating cells of the inner enamel epithelium. At more advanced stages, Notch2 was expressed in the enamel-producing ameloblasts, while it was absent in mesenchyme-derived odontoblasts that synthesize the dentin matrix. Although Notch2 was not expressed in the pulp of adult intact teeth, it was reexpressed during dentin repair processes in odontoblasts and subodontoblastic cells. Transforming growth factor beta-1, which stimulates odontoblast differentiation and hard tissue formation after dental injury, downregulated Notch2 expression in cultured human dental slices, *in vitro*. These observations are consistent with the notion that Notch signaling is an important element in dental physiological and pathogenic conditions.

Introduction

Notch signaling is an evolutionarily conserved mechanism that enables adjacent cells to adopt different fates [1]. In *Drosophila*, the *Notch* gene encodes a transmembrane receptor with a large extracellular domain carrying multiple epidermal growth factor-like repeats and a cytoplasmic domain required for signal transduction [1]. Four Notch proteins (Notch1, Notch2, Notch3, and Notch4) have been identified in vertebrates, while five membrane-bound proteins (Delta1, Delta2, Delta4, Jagged1, and Jagged2) have been recognized as Notch ligands [1–4]. Signals exchanged between neighboring cells through the Notch receptors influence differentiation, proliferation, and apoptotic events at all stages of development, thus controlling organ formation and morphogenesis [1,5]. In vertebrates, mutations in the Notch receptors result in developmental abnormalities and neoplasias [6,7]. Notch malfunction has been shown to disrupt aspects of neurogenesis, somite formation, angiogenesis, and kidney and lymphoid development [8–12]. In humans, constitutively active aberrant forms of Notch are associated with leukemia and solid tumors [13–16]. Furthermore, human congenital syndromes known as CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) and Alagille have been associated with mutations in Notch3 and Jagged1, respectively [17–19].

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Despite the significance of Notch signaling in pathology, relatively little is yet known about the activation of Notch signaling in injured adult organs of vertebrates. For example, few studies have shown Notch expression during regeneration and repair of rat spinal cord, chick inner ear, arteries, and rat teeth [20–23]. The tooth is a useful model for examination of the molecular mechanisms involved in gradual cell fate determination and the differentiation of various cell lineages. Tooth development involves a series of sequential and reciprocal interactions between the oral epithelium and cranial neural-crest-derived mesenchymal cells [24]. These interactions gradually transform the tooth primordia into complex mineralized structures with various cell types, among which the epithelial-derived ameloblasts and the mesenchyme-derived odontoblasts synthesize and secrete the organic components of the enamel and dentin, respectively. Previous data have shown that Notch signaling is involved in both development and homeostasis of rodent teeth [23,25–28]. Although the Notch1,-2, and -3 receptors are reexpressed after lesioning of rodent teeth, Notch2 expression in dental pulp was predominant, presenting a distinct expression pattern in cells adjacent to differentiated odontoblasts [23]. To date, there is no available study on Notch expression in developing and adult human teeth, under both physiological and pathological conditions.

In the present study, we examine Notch2 expression in embryonic and adult human teeth (intact, injured, and carious). We also report on the regulation of Notch2 expression by transforming growth factor beta-1 (TGFβ1), which is involved in odontoblast differentiation and dentin formation after dental injury [29,30], in a culture system of human dental slices *in vitro.*

Materials and methods

Antibodies

Preparation and characterization of a rabbit antiserum against mouse Notch2 have already been described [25]. This antiserum was demonstrated to react specifically with Notch2 and does not cross-react with other Notch molecules. A rat antibody raised against human Notch2 (bhN6) was kindly provided by Dr. Spyros Artavanis-Tsakonas (Harvard University, School of Medicine, Boston, MA). This antibody was demonstrated to react specifically with Notch2 in immunohistochemistry and in Western blotting of human tissues, without any cross-reactivity with other Notch molecules [15,16].

Chemicals and culture medium

The Vector Vectastain ABC kit was purchased from Biosys (Compiègne, France). For the preparation of culture medium, Eagle's basal medium (Gibco BRL, Life Technologies, Inc., NY) containing ascorbic acid (50 μg/mL), was supplemented with 2% fetal calf serum, 100 UI/mL penicillin, and 100 μg/mL streptomycin (Roche, Mannheim, Germany). Other chemicals were obtained from Sigma (St. Louis, MO).

Recombinant protein

Recombinant human TGFβ1 (Bio Vision Research Products, Palo Alto, CA) was used for the *in vitro* studies.

Embryonic tissues

Human fetal tissues were obtained from legal abortions. The material comprised teeth from five fetuses (6–20 gestational weeks). The gestational age was estimated from the fetal foot length and from the last menstruation of the mother. Embryos were healthy, and all tissues were macroscopically and microscopically normal. The fetuses were fixed immediately by the obstetrician in 10% buffered formalin for 2 to 5 days according to their size. The maxillary and mandibular processes from 6- to 12-week-old embryos were embedded in Paraplast at 56°C. The samples, ranging in age from 14 to 20 gestational weeks (gw), were decalcified for 3 weeks in formic acid/10% formalin prior to being cryosectioned. Ten-micrometer-thick sections were used for immunohistochemistry. This study was carried out in compliance with French legislation.

Permanent teeth

Permanent intact teeth (premolars and third molars) extracted for orthodontic reasons and carious teeth of 30- to 40-year-old patients were used in this study. The teeth were freshly extracted and used in this study with the patient's informed consent and following an informed protocol approved by the local ethics committee. The extracted teeth were fixed in 10% neutral-buffered formalin for 7 days, demineralized in sodium formiate for 21 days, and then embedded in paraffin wax. They were serially sectioned (6 μm thick) and then processed for immunohistochemistry.

Tooth processing

Two- to 3-mm-wide and 1- to 1.2-mm-deep cavities were realized in intact first premolars scheduled for extraction at the Dental Care Center of Marseille. The pulp chambers were not exposed during the preparation of the cavities. The cavities were restored with a calcium hydroxide product (Dycal; Dentsplay) which was covered by a temporary filling material (IRM; De Trey Dentsplay IG, Zurich, Switzerland). After a postoperative interval of 9 weeks, the teeth were extracted using a local anesthetic.

Teeth with cavities or carious lesions were fixed in 10% neutral-buffered formalin for 7 days, demineralized in sodium formiate for 21 days, and then embedded in paraffin wax.

Culture of dental slices

Molars extracted for orthodontic reasons were immediately cut into three 750-μm-thick slices. Cultures of dental slices were performed as previously described [30]. Briefly, a small polypropylene tube was glued on the dentin close to

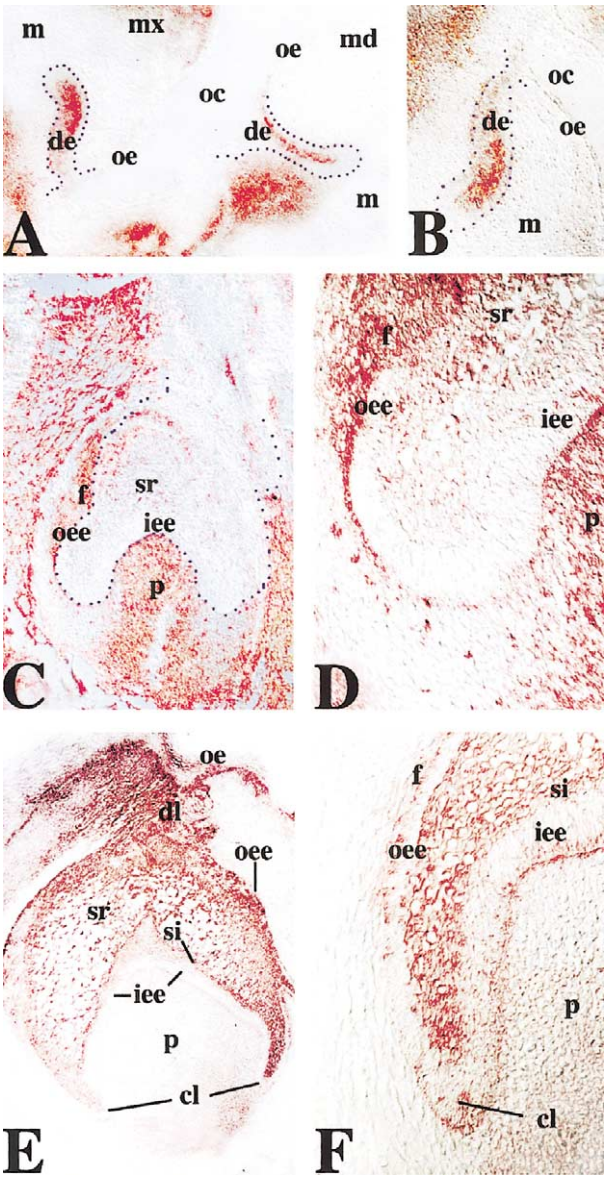


Fig. 1. Immunohistochemical localization of Notch2 in deciduous human teeth at the bud and cap stages of development. Staining is shown in red. Blue dotted lines represent the borders between dental epithelium and mesenchyme. (A, B) At the bud stage, Notch2 is expressed in cells of the dental epithelium (de) that are not in close contact with mesenchymal cells. Mesenchyme (m) around the epithelial bud is negative for Notch2. (C, D) At the early cap stage, strong Notch2 staining is observed in cells of the dental pulp (p) and follicle (f), while a faint labeling is seen in cells of the stellate reticulum (sr) and outer enamel epithelium (oee). The staining was absent in cells of the inner enamel epithelium (iee). (E) At the late cap stage, Notch2 is mainly expressed in cells of the stratum intermedium (si) and stellate reticulum. The staining was absent in pulp cells with the exception of the cervical loop (cl) region where discrete labeling is observed. (F) Higher magnification of E. Note that some cells of the inner and outer enamel epithelia, as well as the basement membrane separating the enamel epithelium from the dental pulp, are also positive for Notch2. Additional abbreviations: dl, dental lamina; md, mandibular process; mx, maxillary process; oc, oral cavity; oe, oral epithelium.

a pulp horn (Fig. 5A). Slices were placed in 12-well culture plates (Falcon, Becton–Dickinson, Oxford, England) and covered with 1 mL of culture medium. Tubes were filled with 50 μL of culture medium supplemented with 20 ng/mL of TGFβ1. Slices were cultured for 3 days without medium change to limit the diffusion of the factor to the nearest pulp horn. TGFβ1-free culture medium was used in control tubes.

Immunohistochemistry

Immunoperoxidase staining of sections was done as previously described. Briefly, the sections were deparaffinized, exposed to a 0.3% solution of hydrogen peroxide in meth-

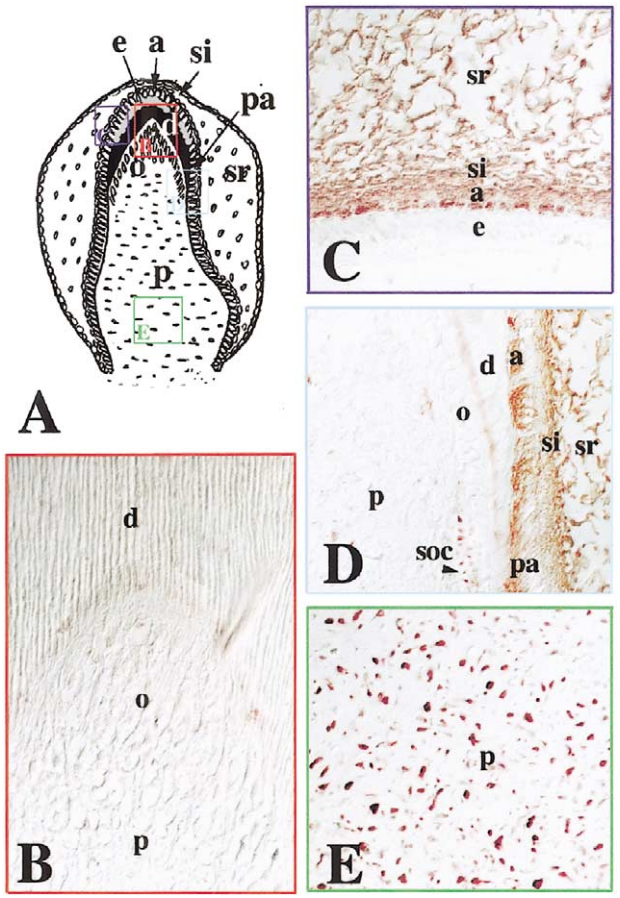


Fig. 2. Immunohistochemical localization of Notch2 in a deciduous human tooth at the bell stage of development. (A) Schematic representation of a tooth at the bell stage. The colored frames show the areas used for the study. (B) The red frame represents the mesenchymal part of the cusp region. Notch2 staining is not observed in functional odontoblasts (o) and pulp (p) fibroblasts. (C) The violet frame represents the epithelial part of the cusp area. Notch2 staining is localized in functional ameloblasts (a), stratum intermedium (si), and stellate reticulum (sr). (D) The light blue frame represents the area where dentin (d) formation starts. In dental epithelium, Notch2 staining is distributed in preameloblasts (pa), ameloblasts, stratum intermedium, and stellate reticulum. In the pulp (p), Notch2 labeling is observed in cells of the subodontoblastic layer (soc), but was absent in differentiated odontoblasts and pulp fibroblasts. (F) Notch2 staining is detected in dental pulp cells of the cervical region. Additional abbreviation: e, enamel.

anol, and then incubated overnight at 4°C with the primary antibody against Notch2. The rabbit Notch2 antibody was incubated at a dilution of 1:500–1:1000 in phosphate-buffered saline (PBS) containing 0.2% bovine serum albumin and 5% normal goat serum. The rat anti-human Notch2 antibody was diluted 1:50 in PBS for cryosections and 1:20 for paraffin sections. Peroxidase was detected by incubation with either 3-amino-9-ethylcarbazole or diaminobenzidine reaction solution. After being stained, the slides were mounted with Aquamount (BDH, Gurr, England). In control sections the primary antibodies were omitted.

Cultured slices were rinsed in PBS and then fixed in 4% paraformaldehyde/PBS solution for 1 h at 4°C. After being rinsed the slices were immersed in 7% saccharose–PBS and then in 15% saccharose–8% glycerol–PBS. The hard tissues were gently discarded and then the pulp tissue was embedded in Tissue Tek compound and immersed in liquid-nitrogen-cooled isopentane (EMS, Washington, PA). Cryostat serial sections (10 μm thick) were collected on 3-aminopropyltriethoxysilane-coated slides and air-dried. Immunoperoxidase staining of sections was done as described above.

Results

Notch2 expression in the developing deciduous human tooth germs

In 5-week-old human embryos, the oral epithelium proliferates into the subjacent mesenchyme and forms a series of epithelial ingrowths into the neural-crest-derived mesenchyme at sites corresponding to the position of the future deciduous teeth. From this point tooth development proceeds in three descriptive stages: the bud, cap, and bell stages. At the 6th gestational week (gw) (Fig. 1A), the epithelial ingrowth gives rise to the epithelial dental bud. At this stage, immunoreactivity for Notch2 was observed in dental epithelial cells that were not in contact with mesenchymal cells. Notch2 staining was absent from mesenchymal cells that surround the tooth bud (Figs. 1A and 1B). During the early cap stage of development (8–10 gw), the dental epithelium forms the enamel organ and the mesenchyme forms the dental papilla and dental follicle. At this stage, heavy Notch2 labeling was observed in mesenchymal cells of the dental papilla and follicle (Figs. 1C and 1D). In the enamel organ, the staining was absent in cells that were not in contact with dental mesenchymal cells, while other epithelial cells exhibited a moderate immunoreactivity. During the late cap stage (12–14 gw), the enamel organ is formed by four distinct cell layers: the inner enamel epithelium, the outer enamel epithelium, the stellate reticulum, and the stratum intermedium. Notch2 labeling was detected in cells of the stellate reticulum and stratum intermedium, whereas the staining was faint in cells of the inner and outer enamel epithelia (Figs. 1E and 1F). A weak Notch2 reactivity was also found in some cells of the dental papilla and dental follicle.

Continued growth of the tooth germ leads to the bell stage of tooth development. Dentinogenesis is initiated at the tip of the cusp and the tooth shape (crown morphology) is apparent (Fig. 2A). The pulp cells adjoining the dental epithelium differentiate into odontoblasts and start to secrete the organic matrix of dentin. Inner enamel epithelial cells differentiate into preameloblasts/ameloblasts, which are synthesizing the enamel matrix proteins. At this stage (20 gw), Notch2 was expressed in ameloblasts as well as in cells of the stellate reticulum and stratum intermedium (Figs. 2C and 2D). In dental papilla, Notch2 immunoreactivity was not observed in odontoblasts (Fig. 2B). A staining gradient was observed in pulp fibroblasts from the cervical loop to the cusp region: cells in the cervical loop were positive (Fig. 2E), while the immunostaining decreased toward the cusp region (Figs. 2A and 2D). Similarly, the distribution of Notch2 in cells of the subodontoblastic layer exhibited a gradient following their maturation state: Notch2 immunoreactivity was found in young cells during the initiation of dentin formation (Fig. 2D), but was absent in mature cells (Figs. 2B and 2D).

Notch2 expression in carious human teeth

Notch2 immunoreactivity was completely absent in adult intact teeth (data not shown), but staining was observed in adult carious teeth. Notch2 staining was detected in the carious front level, in the bacteria that have infiltrated the dentin (Fig. 3A). In dental pulp, staining was observed in odontoblasts situated beneath the carious front and in cells of the blood vessels (Figs. 3B and 3C). The pulp fibroblasts were negative for Notch2 (Figs. 3B and 3C). In response to an increased irritation, the degenerated odontoblasts are replaced by newly formed odontoblast-like cells, which elaborate the reparative dentin. Disintegrated odontoblasts facing the lesion and blood vessels were negative for Notch2 (Fig. 3D), while Notch2 staining was observed in cells situated in the proximity of blood vessels (Fig. 3D).

Notch2 expression in the mature permanent human teeth after injury

Nine weeks after cavity preparation, odontoblasts facing the injury site produce either reactionary or reparative dentin (Figs. 4A–4C). Reactionary dentin matrix is synthesized by odontoblast-like cells replacing the dying odontoblasts after the injury [22,29]. Notch2 immunoreactivity was not detected at the site of the reactionary dentin production (Fig. 4D), but was evident at a distance from the cavity preparation (Fig. 4E). Notch2 staining was absent from odontoblasts (Fig. 4C), but cells of the subodontoblastic layer exhibited a strong signal. Immunoreactivity for Notch2 was also observed in blood vessels of the pulp (Figs. 4D–4H).

TGFβ1 down-regulates Notch2 expression in human dental pulp cells in vitro

It has been shown that TGFβ1 is involved in odontoblast differentiation and early steps of dentin matrix synthesis *in*

vitro [29,31], suggesting that this molecule may affect Notch2 expression in human dental pulp after injury. To test this, we placed small tubes releasing TGFβ1 onto the dentin of cultured thick-sliced human teeth (Fig. 5A) and followed the expression of Notch2 by immunohistochemistry. Analysis of the slices showed Notch2 expression in odontoblasts and cells of the subodontoblastic layer located far away from the TGFβ1 source (Figs. 5B and 5C). Staining was also observed in blood vessels of the pulp (Fig. 5C). This expression pattern of Notch2 was similar to that observed in control slices (cultured in the presence of TGFβ1-free tubes; data not shown). Notch 2 staining was absent in odontoblasts, subodontoblastic cells (Fig. 5D), and blood vessels (Fig. 5E) located near the TGFβ1 source (Figs. 5D and 5E). At a more distant area (i.e., between the pulp horns), Notch2 reactivity was observed in cells of the subodontoblastic layer but was absent from odontoblasts (Fig. 5F), indicating a dose-dependent effect of TGFβ1 on Notch2 expression in pulp.

Discussion

Notch signaling controls cell fate commitment during development of a wide range of tissues and organs [1,5]. In *Drosophila*, Notch is expressed in cells that are not yet terminally differentiated and is absent from adult tissues, with the exception of the ovaries and testes [1]. Here, we have examined the expression of the Notch2 receptor in embryonic, intact, and injured adult human teeth. We found a dynamic pattern of Notch2 expression in human dental tissues during embryonic development that closely resembles the expression patterns previously reported in rodents [25,27]. Moreover, we have shown that intact adult human teeth were devoid of Notch2 expression, but that its expression was induced in dental pulp under pathological conditions such as carious lesions and dental wound healing, reflecting the involvement of Notch signaling in reparative processes.

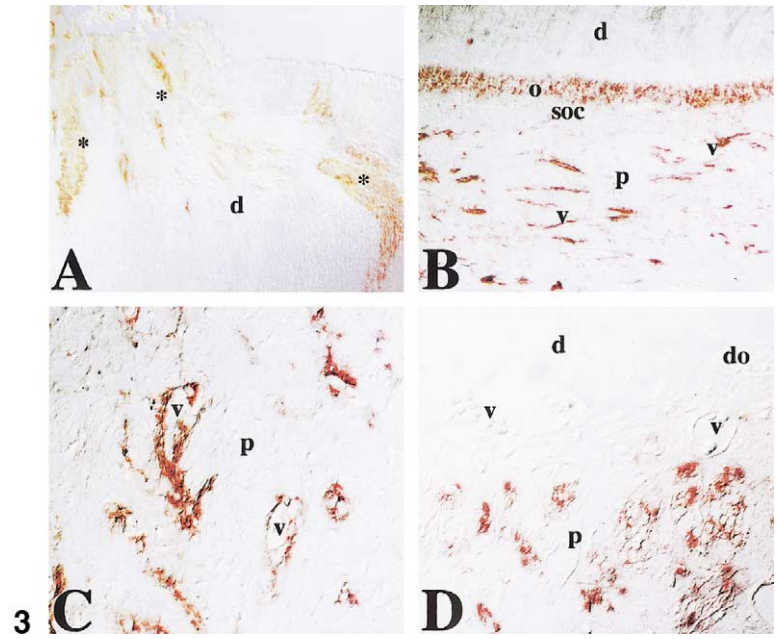
Notch2 in developing and intact adult human teeth

Notch2 is expressed at all stages of the developing human teeth (bud, cap, and bell stages). Epithelial–mesenchymal shifts of Notch2 expression during tooth development and Notch2 downregulation in mature dental tissues indicate the similarities that exist in the expression patterns of Notch2 between human and rodent teeth. However, the pattern of Notch2 expression in the epithelium of the developing human teeth differs from that previously reported in the teeth of rodents. Notch2 is expressed in preameloblasts and ameloblasts of deciduous human teeth, whereas Notch2 expression is not observed in these cells of rodent teeth [25,27,28]. The expression of Notch receptors in ameloblasts reported here suggests that Notch signaling plays a role in later developmental stages of ameloblast maturation after lineage commitment. The role of Notch in terminally differentiated cells is not known, but it has been speculated that Notch may confer onto these cells some degree of developmental plasticity [1,5,32–35]. In addition to expressing Notch2 receptors, ameloblasts may also express the ligands Jagged1, Jagged2, and Delta1, as has been previously reported in rodent teeth [26–28,36]. It has been shown that binding of one of these three ligands to Notch2 induces cleavage and nuclear translocation of the receptor [37]. These data imply that Notch signaling during ameloblast differentiation and/or maturation may be mediated not only by heterotypic interactions between ameloblast precursors (i.e., inner dental epithelial cells) and the neighboring cells (i.e., cells of the stratum intermedium), but also by homotypic interactions between ameloblast precursors themselves. The reason for the species-specific difference in Notch2 expression in teeth is not yet known, but it may be the difference in enamel structure between rodents and human.

During odontoblast differentiation, Notch2 expression by mesenchyme cells may suggest the presence of still-undifferentiated cells that are probably committed to an odontoblastic fate [23,38]. Notch2 expression in the pulp follows a cytodifferentiation gradient: expression in subodontoblastic cells of the apical area becomes progressively downregulated in the cusp area where pulp cells have completed their maturation. Notch2 is not the only protein of the Notch family that is expressed in the pulp of rodent teeth: Notch1 and Notch3 are also expressed in cells of the subodontoblastic layer [25], thus suggesting that Notch2 may form heterodimers with Notch1 and/or Notch3. However, only

Fig. 3. Immunohistochemical localization of Notch2 in sections of carious permanent human teeth. (A) Notch2 staining is observed in bacteria (asterisks) that have infiltrated the dentin (d). (B) In the pulp of carious teeth, Notch2 reactivity is found in odontoblasts (o) facing the carious irritation as well as in blood vessels (v). The staining was absent in cells of the subodontoblastic layer (soc) and pulp (p) fibroblasts. (C) Higher magnification of the pulp of a carious tooth showing Notch2 labeling in cells of the dilated blood vessels. (D) Notch2 immunoreactivity is absent in disintegrating odontoblasts (do) and blood vessels situated beneath the carious front. A staining is seen in inflammatory cells of the pulp.

Fig. 4. Immunohistochemical localization of Notch2 in sections of permanent human premolars after cavity preparation. (A) Schematic illustration of a premolar showing reactionary dentin production, 9 weeks after cavity preparation (cav). Colored frames indicate the areas shown in the following panels. (B) Hematoxylin–eosin staining. Nine weeks after the cavity preparation, reparative dentin (rd) is seen beneath the injury site. (C) In injured teeth, absence of bacterial infiltration (deep violet color) of the dentin (d) is demonstrated after a Brown and Brenn (gram) staining for tissues. Tissues are yellow in color. (D) Notch2 reactivity is absent in odontoblasts and pulp cells located near the injury site. Note the positive staining of blood vessels (v). (E) At a small distance from the injury site, Notch2 staining is distributed in cells of the subodontoblastic layer (soc) and blood vessels. Odontoblasts remain negative for Notch2. (F–H) Notch2 labeling is observed in cells of the subodontoblastic layer and in blood vessels at sites far away of the injury area. Note the absence of the staining in odontoblasts and pulp (p) fibroblasts.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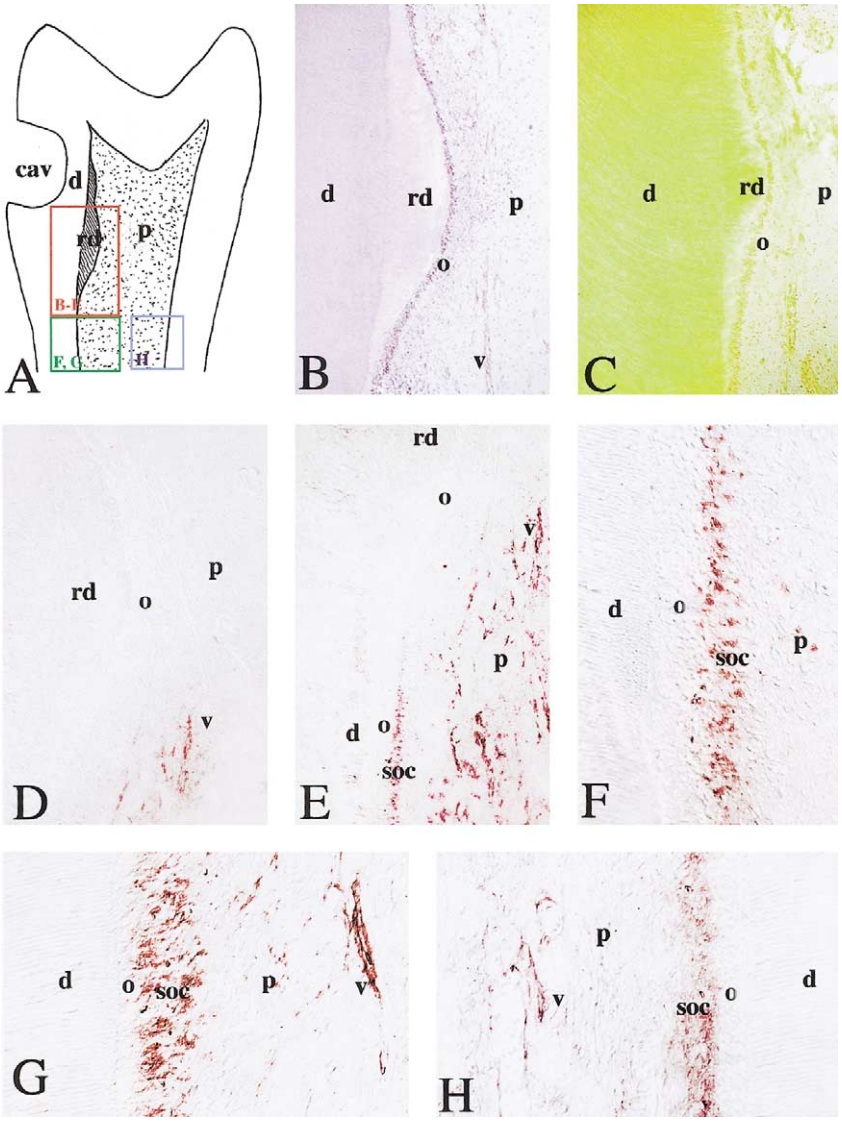
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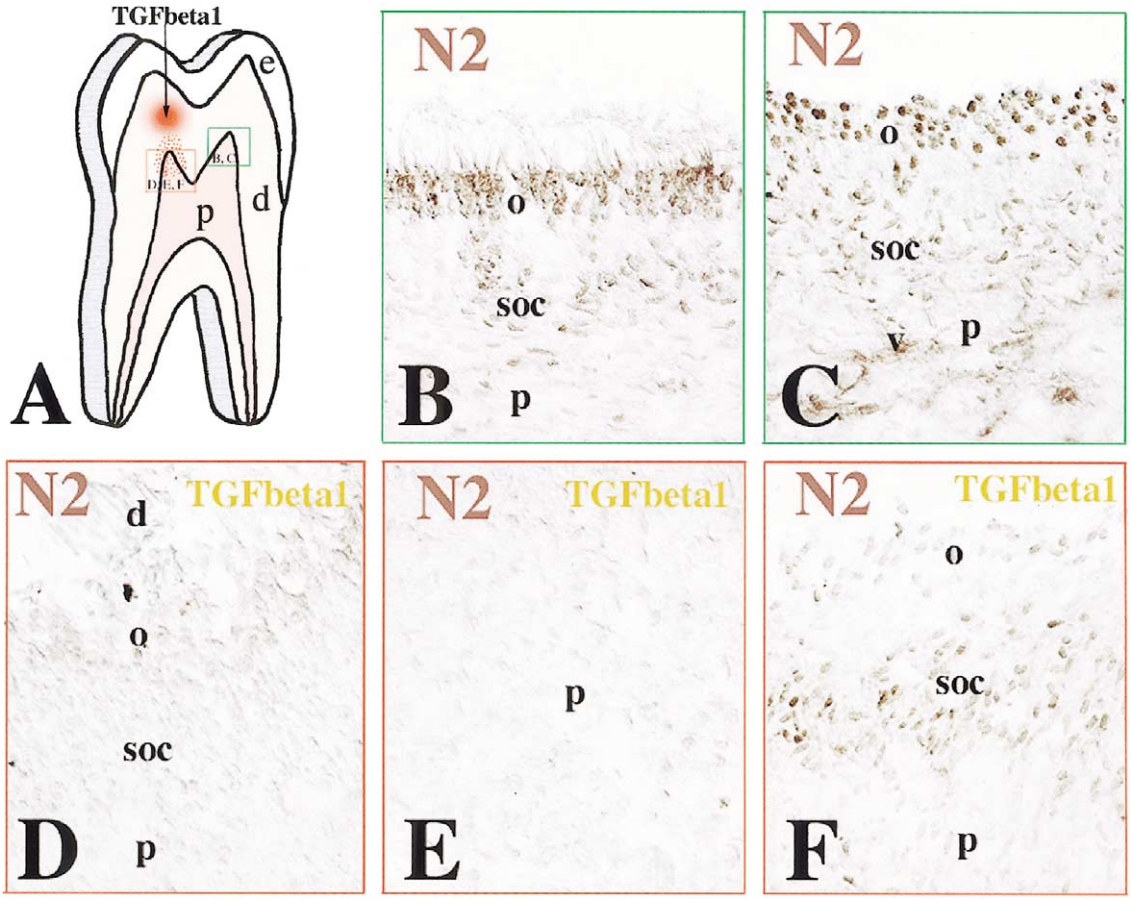

Fig. 5. Effects of TGFβ1 on Notch2 expression in cultured human tooth slices *in vitro*. (A) Schematic representation of the human tooth slice culture system. The red spot shows the area where the TGFβ1-containing polypropylene tube was placed, thus permitting the diffusion of the molecule (small red dots) to the dental pulp. Frames indicate selected areas situated either near the TGFβ1-source (red frame) or at a distance from the source (green frame). (B) Notch2 staining is localized in odontoblast (o) and subodontoblastic cells (soc) that are not under the influence of TGFβ1 signaling. Note the absence of staining in odontoblastic processes. (C) Nuclear Notch2 staining is detected in odontoblasts that lost their processes. Weaker labeling is observed in the underlying subodontoblastic cells and in blood vessels (v). (D, E) Notch2 reactivity was absent in odontoblasts, subodontoblastic cells, and blood vessels located near the TGFβ1-stimulated area. (F) At the margin of the diffusion zone, Notch2 staining is located only in subodontoblastic cells, while odontoblasts remained negative for Notch2. Additional abbreviations: d, dentin; e, enamel; p, pulp.

Notch2 is detected in cells of the subodontoblastic layer in human teeth (our unpublished results).

Notch2 in adult human teeth under pathological conditions

The pulp represents a pool of undifferentiated cells that can give rise to odontoblasts, cells of the subodontoblastic layer, and pulp fibroblasts [23,24,28,31]. The molecular mechanism by which these cells are kept undifferentiated remains poorly understood. The importance of the cell–cell interaction in the dental pulp environment has been well recognized, and in this context, expression of Notch receptors in cells of the subodontoblastic layer and expression of ligands in odontoblasts are of particular interest. When odontoblasts undergo apoptosis after a dental injury, mesenchymal cells of the pulp differentiate into odontoblastic-like cells that will form tertiary dentin [31]. The present data confirm previous findings in irritated rodent teeth [23] and suggest that the Notch signaling pathway is involved in the dynamic processes triggered by pulp injury. Notch2 receptors in injured human adult teeth are most likely utilized by early odontoblast precursors and intermediate-stage cells of the subodontoblastic layer, but not by terminally differentiating odontoblasts, suggesting a role for the Notch pathway in enhanced survival of uncommitted precursors, while preserving multilineage potential. In line with this interpretation, previous studies in adult mammalian tissues also concluded that Notch expression is associated with cells that represent a proliferating precursor pool dedicated to a specific fate [15,16,39].

Notch2 expression was not only activated in pulp cells close to the injury (coronal part of the tooth), but also at a distance (dental roots), suggesting that important pools of odontoblast progenitors are located in the roots. Notch2 activation in pulp cells located far away from the lesion may demonstrate the ability of Notch to influence their prolifer-

ation and/or migration at the injury site. It has been demonstrated recently that Notch signaling regulates keratinocyte spreading and motility [40]. Moreover, a link between proliferation events and Notch has been seen in several instances [41]. However, the effect of Notch seems to be indirect, since the regions of the highest Notch activity do not always coincide with the regions of the highest mitotic activity. Notch2 expression was also activated in vascular structures of the irritated human pulp, reflecting either ingrowth of new blood vessels and/or inflammatory reactions [11,32,42].

Activation of the Notch2 receptor in odontoblasts of carious teeth is correlated with apoptosis rather than differentiation. In mice, Notch2 deficiency correlates with earlier onset and higher incidence of apoptosis [43]. Furthermore, Notch activation during cell maturation has generally been associated with enhanced survival and protection against apoptosis. Notch activation appears to inhibit apoptosis in the thymus and in erythroleukemia cells [44,45]. Thus, the oncogenic forms of the vertebrate Notch homologues may reflect an inhibition of cell death rather than a stimulation of proliferation. However, several studies have demonstrated that Notch may have an opposite effect by inducing apoptosis in other cell types such as B cells and neurogenic precursors [46,47]. We have shown recently that, in human teeth, odontoblasts underlying a carious front are eliminated by apoptosis (results submitted for publication). Notch2 upregulation in odontoblasts facing the carious lesion may suggest an additional role for the Notch signaling in apoptotic events occurring in teeth under pathological conditions.

Regulation of Notch2 expression in dental pulp

In order to influence developmental decisions, Notch must obviously interact with other signaling pathways. Several studies have demonstrated that TGFβ and FGF molecules can modulate Notch signaling [26–28,48,49]. Notch-dependent cell fate acquisition between nonequivalent dental precursor cells could be influenced by such extrinsic signals. Signaling molecules of the TGFβ superfamily seem to be important for hard tissue formation after pulp injury. During tooth formation, TGFβ1 stimulates odontoblast differentiation and dentin matrix synthesis [29,31]. TGFβ1 may also induce proliferation and migration of subodontoblastic cells and pulp fibroblasts [30]. TGFβ1 downregulated Notch2 expression in pulp cells of the *in vitro* cultured thick-sliced human teeth. This is in agreement with previous findings showing that TGFβ acts as an antagonist to the Notch4 action [49]. Furthermore, we have previously reported that the expression of the Notch ligand Delta1 in dental mesenchyme is upregulated by TGFβ1 [27]. These results indicate that dental cell lineage restriction is under the concomitant control of the Notch and TGFβ1 pathways.

A variety of studies have shown that deregulation of Notch signaling, either by deregulation of Notch expression or by expression of mutated forms, is associated with different types of syndromes and neoplasias [1,6,7]. The present study shows that elevated levels of Notch2 are also associated with responses to dental injury and carious lesions. The data indicate that properly regulated activation of the Notch pathway is important for controlling cell fate choices during development and maintaining the correct balance among cell proliferation, differentiation, and apoptosis in the adult organism.

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