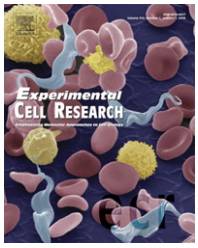
EXPERIMENTAL CELL RESEARCH 314 (2008) 869–877

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/yexcr



Research Article

Apoptosis in developmental and repair-related human tooth remodeling: A view from the inside

Thimios A. Mitsiadis[a,*], *Cosimo De Bari*[b], *Imad About*[c]

[a]*Institute of Oral Biology, Department of Orofacial Development and Structure, Faculty of Medicine, University of Zurich, Plattenstrasse 11, 8032 Zurich, Switzerland*
[b]*Department of Medicine & Therapeutics, University of Aberdeen, Aberdeen AB25 2ZD, United Kingdom*
[c]*Laboratoire IMEB, Faculté d'Odontologie, Université de la Méditerranée, 27 Boulevard Jean Moulin, 13385 Marseille Cedex 5, France*

ARTICLE INFORMATION

Article Chronology:
Received 3 August 2007
Revised version received
2 November 2007
Accepted 3 November 2007
Available online 12 November 2007

Keywords:
Apoptosis
Odontoblasts
Tooth
Human
Injury

ABSTRACT

Apoptosis is a key phenomenon in the regulation of the life span of odontoblasts, which are responsible for dentin matrix production of the teeth. The mechanism controlling odontoblasts loss in developing, normal, and injured human teeth is largely unknown. A possible correlation between apoptosis and dental pulp volume reduction was examined. Histomorphometric analysis was performed on intact 10 to 14 year-old premolars to follow dentin deposition and evaluate the total number of odontoblasts. Apoptosis in growing healthy teeth as well as in mature irritated human teeth was determined using a modified TUNEL technique and an anti-caspase-3 antibody. In intact growing teeth, the sequential rearrangement of odontoblasts into a multi-layer structure during tooth crown formation was correlated with an apoptotic wave that leads to the massive elimination of odontoblasts. These data suggest that apoptosis, coincident with dentin deposition changes, plays a role in tooth maturation and homeostasis. Massive apoptotic events were observed after dentin irritation. In carious and injured teeth, apoptosis was detected in cells surrounding the lesion sites, as well as in mono-nucleated cells nearby the injury. These results indicate that apoptosis is a part of the mechanism that regulate human dental pulp chamber remodeling during tooth development and pathology.

Introduction

Apoptosis is a genetically regulated form of cell death that is implicated in biological processes ranging from embryonic development to aging, from normal tissue homeostasis to various human diseases [1,2]. Genetic evidence has identified both positive and negative regulators of apoptosis [3,4]. Initiators of apoptosis include, between others, ultraviolet irradiation and deprivation of survival factors such as cytokines. These stimuli in turn generate a characteristic pattern of gene expression. The molecular machinery of apoptosis is evolutionarily conserved and intrinsic to all metazoan organisms. The principal effectors are a family of proteases termed caspases [5]. Apoptosis is characterized by morphological and ultrastructural changes that include chromatin condensation, nuclear fragmentation, cell rounding and shrinkage,

* *Corresponding author*. Thimios Mitsiadis, Institute of Oral Biology, University of Zurich, Plattenstrasse 11, 8032 Zurich, Switzerland. Fax: +41 446344310.
E-mail address: thimios.mitsiadis@zzmk.uzh.ch (T.A. Mitsiadis).

0014-4827/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexcr.2007.11.001

leading to cell breakage into apoptotic bodies that are rapidly phagocytozed and digested by macrophages or by neighboring cells [6].

During development, apoptosis eliminates individual unnecessary cells as well as entire vestigial structures. It has been shown that apoptosis plays a key role in tooth shape and size [7–10]. Apoptosis is also involved in tooth anomalies associated with cleft lip and palate [11]. In a dynamic interplay of cell proliferation and cell death, the developing tooth retains precisely the type and number of cells needed to proceed into maturity. It is admitted that odontoblasts are terminally differentiated cells that survive as long as the integrity of the tooth is preserved [12,13]. However, the dental pulp volume decreases gradually on ageing due to the continuous production of dentin matrix by odontoblasts. This age-related pulp chamber reduction is associated with the elimination of a certain number of odontoblasts by apoptosis [14,15]. In pathological conditions involving mild carious lesions, odontoblast activity is stimulated to elaborate a reactionary dentin [16–20]. In contrast, dental irritations involving violent stresses (i.e. cavity preparations) lead to odontoblast disintegration and newly formed odontoblast-like cells elaborate a reparative dentin. Reparative dentinogenesis involves either necrosis or apoptosis of odontoblasts. Reactionary dentinogenesis results from a stimulation of existing odontoblasts and this process takes place in the absence of cell death. However, the deposition of reactionary dentin decreases the pulp chamber volume and could thereafter favor apoptosis of odontoblasts in a comparable way to the age-related apoptosis.

The study of apoptosis in growing and injured human teeth is important for at least two reasons. First, as odontoblasts must survive for the entire life span of the animal in spite of the continuous decrease of the dental pulp chamber volume [12,13], an understanding of the regulatory mechanisms that control cell death in dental pulp may provide new insights into the process of tooth homeostasis. Second, conditions or agents that initiate apoptosis (i.e. bacterial infection, pulp ischemia, mechanical stimuli, dental restorative products) trigger dental disorders accompanied by dentin remodeling [19,20]. Using an *in situ* end-labeling assay to detect cell death, we define a precise time period during tooth maturation when apoptosis is stimulated in the odontoblastic layer. The location and extent of this process helps to explain the differences in size and appearance of the adult dental pulp chamber. Furthermore, concentrated cell death at the injury and carious sites suggests an additional role for apoptosis in dentin remodeling that is related to pathological conditions. These findings improve our understanding on the mechanisms involved in tooth maturation and tooth-related diseases in humans.

Materials and methods

Preparation of teeth

For a first set of experiments, 20 premolars of 10 to 14 year-old patients were used. The premolars were composed of 4 groups of 5 teeth each according to their age (10, 11, 12.5 and 14 years old). These teeth were used to determine the number of odontoblasts per 100 μm and examine apoptosis.

In another experimental set, cavity preparations were performed as previously described in 17 premolars of 11 to 12 year-old patients [15], while 4 intact premolars were used as a control. A dental product (IRM) was used to restore the cavities, in conjunction with a calcium hydroxide lining material (Dycal, Dentsply, Milford, DE, USA). The teeth were extracted after a post-operative interval of 8 to 9 weeks. Finally, 10 carious third molars of 30 to 40 year-old patients were used. No radiographic or clinical indication of irreversible pulp reaction was seen in these teeth.

Immediately after extraction, the roots of the teeth were sectioned in order to obtain a quicker fixation. Since fixation plays a major role in triggering anoxic apoptosis [21], 4% neutral formol was used for 5 days to minimize the risk of false results induced by a long-term fixation. After demineralization with 10% formic acid, 5 μm serial sections were performed. Two intact teeth were fixed with 4% paraformaldehyde at 4 °C for 3 days and then were sliced into 50 μm sections without a previous demineralization. Hematoxylin–eosin staining was performed every other section of decalcified normal human teeth.

Histomorphometric and statistical analysis

Hematoxylin–eosin staining was performed on alternate sections of decalcified normal human teeth. The external and internal dentin perimeters were measured as described previously [15]. On the same sections, nuclei of the odontoblasts per 100 μm were recorded in 12 automatically pre-selected fields at the cement–enamel junction area. Statistic analysis was performed as described previously [15].

TUNEL assay

A method for identifying and quantifying apoptosis is the detection of fragmented DNA by terminal transferase mediated dUTP-digoxigenin nick end labeling (TUNEL). Apop Tag® detection kits with either fluorescein- or peroxidase-conjugated antibodies were used (Oncor, Ind., Gaithersburg, MD, USA). The TUNEL assay was performed as previously described [22]. Briefly, the sections were treated with the terminal deoxyribonucleotidyl transferase (TdT, Gibco-BRL) for 1 h at 37 °C. The demineralized sections were incubated with the peroxidase-conjugated anti-digoxigenin antibodies for 30 min at room temperature, treated with diaminobenzidine (DAB, Sigma) for 6 min, and then were counterstained with methyl green. The 50 μm thick sections were incubated with fluorescein-conjugated anti-digoxigenin antibodies (Vector Laboratories, Burlingame, CA) for 12 h at 4 °C. The slides were then incubated with 5 μg/ml propidium iodide (Molecular Probes, Eugene, OR) and 100 μg/ml RNAse A (Sigma) for 30 min at 37 °C. Slides were washed, mounted with Vectashield (Vector Laboratories, Burlingame, CA), and observed by epifluorescence. Sections of rat mammary glands were included in each experiment as positive controls, and negative controls