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Original Article

Caspase-7 participates in differentiation of cells forming dental hard tissues

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Apoptosis during tooth development appears dependent on the apoptotic executioner caspase-3, but not caspase-7. Instead, activated caspase-7 has been found in differentiated odontoblasts and ameloblasts, where it does not correlate with apoptosis. To further investigate these findings, the mouse incisor was used as a model. Analysis of caspase-7-deficient mice revealed a significant thinner layer of hard tissue in the adult incisor. Micro computed tomography scan confirmed this decrease in mineralized tissues. These data strongly suggest that caspase-7 might be directly involved in functional cell differentiation and regulation of the mineralization of dental matrices.

Key words: ameloblast, apoptosis, caspase, cell differentiation, odontoblast.

Introduction

Caspase-7 is a cystein protease involved in apoptosis and inflammation. Caspase-7 has often been considered to be redundant to caspase-3, another member of the death trio of executive caspases. Both of these enzymes become activated by cleavage, mediated by a cascade initiated by a death ligand–receptor interaction or by apoptosome formation after cytochrome-c release from the mitochondria (Lamkanfi & Kanneganti 2010). In addition, particularly caspase-7 has been linked to inflammatory responses (Martinon & Tschopp 2007). Recently, non-apoptotic and non-inflammatory functions of caspases have been suggested, such as a role in tissue regeneration, cell fate determination, neural activation and cell differentiation (Kuranaga 2012). Caspase-3, the most studied caspase, was demonstrated to be a putative gatekeeper in stem cells, playing a role in non-apoptotic events in several stem cell derived lineages (Fujita *et al.* 2008). Lack of caspase-3 causes disorders also in bone turnover due to decreased osteogenic differentiation of bone marrow stem cells (Miura *et al.* 2004).

Morphogenesis of tooth germs during odontogenesis is accompanied by proliferation, differentiation, adhesion, migration, and also apoptosis (Peterkova *et al.* 2003; Matalova *et al.* 2004). Caspases become activated during the course of tooth development (Matalova *et al.* 2006; Setkova *et al.* 2007), particularly in relation to apoptotic events (Matalova *et al.* 2012a). Notably, non-apoptotic cleavage of caspase-3 was reported in normal and neoplastic odontogenic epithelial cells in human patients (Kumamoto *et al.* 2001). Moreover, cleaved caspase-7 was found in mouse molars in the primary enamel knot undergoing apoptosis but also in non-apoptotic areas (Matalova *et al.* 2012b). Attempts were thus made to further investigate the possible non-apoptotic role of caspase-7, by moving to the mouse incisor as a model.

The mouse incisor grows throughout the animal's life with the population of hard tissue producing cells (odontoblasts and ameloblasts) renewed by differentiation of cells derived from populations of stem cells located at the apical base of the tooth. The epithelial stem

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cells are housed in a bulge at the end of the labial cervical loop, while the mesenchymal stem cells have been suggested to reside distally near from the cervical loop (Harada *et al.* 1999; Lapthanasupkul *et al.* 2012). The mouse incisor forms a tooth bud around embryonic day (E) 13, enters the cap stage (E14–15) and forms a bell structure by E16 (Kieffer-Combeau *et al.* 2001). During embryonic development, the tooth germ elongates in a posterior direction due to cell proliferation. The elongation then proceeds anteriorly and allows tooth eruption and lifelong compensation for the progressive abrasion at the incisor tips. The labial and lingual parts of the incisor differ in histology, as only the labial side is covered by enamel, allowing the upper and lower incisors to self-sharpen on contact. The mouse incisor, therefore, represents a unique model for addressing questions of growth and differentiation, allowing several stages of cell differentiation to be visualized as gradients on a single sagittal section.

This work aims to investigate the possible non-apoptotic functions of caspase-7 in cell differentiation during odontogenesis by evaluation of the cleavage (activation) pattern of caspase-7, and analysis of the phenotype of loss of caspase-7 on formation of the continuously growing mouse incisor.

Materials and methods

Samples

CD1 mice were killed according to the experimental protocol approved by the Laboratory Animal Science Committee of the IAPG CAS, v.v.i., Brno, Czech Republic. Mouse heads or mandible quadrants (at postnatal stages) were immediately fixed in 4% buffered paraformaldehyde and decalcified in buffered ethylenediaminetetraacetic acid (EDTA). Samples were dehydrated through a graded series of ethanols, treated with xylene, paraffin embedded and cut in 4-μm serial sections. Prenatal (embryonic) stage E17.5, postnatal (P) stages P0, P5 and P10 were used in the study. Caspase-$7^{-/-}$ mice that had been backcrossed on the C57BL/6 genetic background over at least 10 generations were provided by Professor P. Vandenabeele (Duprez *et al.* 2011) and samples processed as for wild type animals.

Immunohistochemistry

Immunohistochemistry was performed as described in Matalova *et al.* (2012b). Briefly, deparaffinized and rehydrated histological sections were pre-treated (only anti-caspase-7 samples) in citrate buffer (pH = 6.0) 10 min/98°C and in 3% hydrogen peroxide in PBS (5 min/RT). The primary antibody (anti-caspase-7: Cell Signaling 9491, anti-amelogenin: Abcam ab59705, anti-PCNA: Santa Cruz sc-7907) was diluted (anti-caspase-7: ready-to-use solution 50x, anti-amelogenin: ready-to-use solution 400x, anti-PCNA: 100×), and applied overnight/4°C. Peroxidase-conjugated streptavidin-biotin system (Vectastain, S7100) and chromogen substrate diaminobenzidine (DAB, Dako, K3466) reactions were used to visualize positive cells as brown. Slides were counterstained by hematoxylin to clearly distinguish the cell nuclei. Negative control was performed by omitting the primary antibody. Specificity of the selected anti-caspase-7 antibodies was verified using caspase-7 deficient tissues.

MicroCT

Specimens for microCT (computed tomography) were scanned using a GE Locus SP microCT scanner. The specimens were immobilized using cotton gauze and scanned to produce 14 μm voxel size volumes, using an X-ray tube voltage of 80 kVp and a tube current of 80 μA. An aluminum filter (0.05 mm) was used to adjust the energy distribution of the X-ray source. The specimens were characterized further by making three-dimensional isosurfaces, generated and measured using Microview software (GE). Mineral measurements were carried out using the Advanced Bone Module of Microview. Scan data was reconstructed and rendering carried out using the Microview 2.2 software package. Dentin and enamel was segmented using Microview's auto-thresholding facility and isosurfaces were reconstructed separately and loaded into the software using the Geometry overlay feature. Statistical significance was determined using a *t*-test and *F*-test ($P > 0.05$). Eight adult specimens were analyzed, four wild type (WT) mice and four knock-out (KO) mice.

Results

Cleaved caspase-7 during incisor development

At E17.5, the incisor tooth germ has reached the bell stage. At this stage the differentiation of functional odontoblasts proceeds as a gradient from the apex (cervical loops), while the ameloblasts are just starting to differentiate (Fig. 1A). Positive staining for cleaved caspase-7 was apparent particularly in the cytoplasm of the differentiating ameloblasts, cells of the stratum intermedium and also the polarized odontoblasts (Fig. 1B). Staining was detected on the inner portion of the epithelium on the labial part of the cervical loop (Fig. 1C). In contrast, no expression was observed on the lingual part of the cervical loop (data not shown).

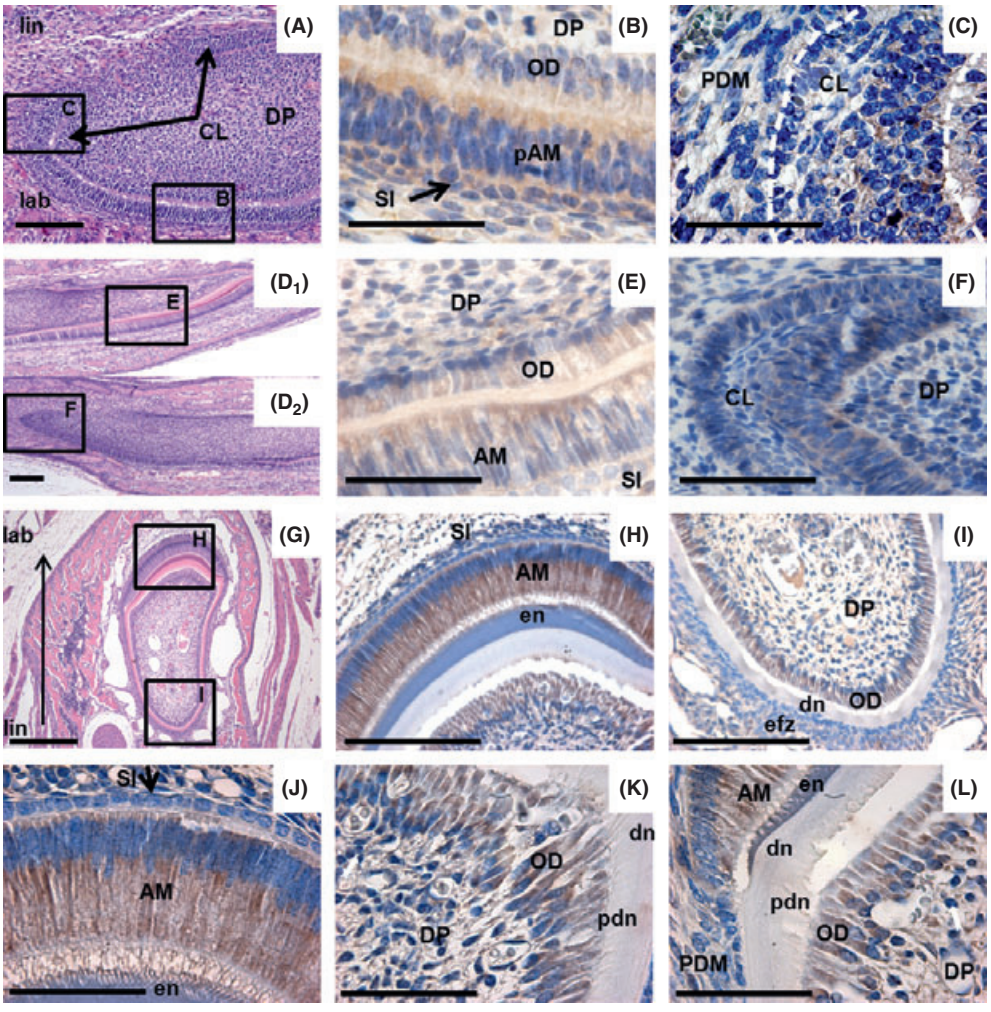

Fig. 1. Cleaved caspase-7 in incisor development. (A,D,G) Hematoxylin/Eosin stained tissue. (B,C, E, F, H, I) Immunohistochemistry of cleaved caspase-7. (A–C) Embryonic day (E) 17.5. (D–F) Postnatal day (P) 0. (G–I) P10. (A): Sagittal section, with clearly formed cervical loop. Scale bar = 100 μm. (B) Caspase-7 positive ameloblasts, odontoblasts and stratum intermedium cells. Scale bar = 100 μm. (C) Cervical loop at apex of tooth with positive label in the inner epithelium on the labial side. Scale bar = 50 μm. (D1) Sagittal section. Anterior region with differentiated ameloblasts and odontoblasts. (D2) Apical part of the tooth with the cervical loop. Hematoxylin/Eosin. Scale bar = 100 μm. (E) Caspase-7 positive ameloblasts and odontoblasts. Scale bar = 100 μm. (F) Caspase-7 localized to the inner epithelium of the labial cervical loop. Scale bar = 100 μm. (G) Frontal section. Enamel covers the labial part of the tooth (top) while the lingual side (bottom) remains enamel free. Scale bar = 100 μm. (H) Detailed look at the localization of cleaved caspase-7 at P10 with positive ameloblasts and negative stratum intermedium cells. Scale bar = 50 μm. (I) A border is evident between the caspase-7 positive ameloblasts and odontoblasts and negative periodontal mesenchyme and dental pulp. Scale bar = 50 μm. (J) Detailed look at the localization of cleaved caspase-7 at P10 with positive ameloblasts and negative stratum intermedium cells. Scale bar = 50 μm. (K) Detailed look at the localization of cleaved caspase-7 at P10 with positive odontoblasts. Scale bar = 50 μm. (L) A border is evident between the caspase-7 positive ameloblasts and odontoblasts and negative periodontal mesenchyme and dental pulp. Scale bar = 50 μm. AM, ameloblasts; b, bone; CL, cervical loop; dn, dentin; DP, dental papilla; en, enamel; OD, odontoblasts; pAM, preameloblasts; PDM, peridental mesenchyme; pdn, predentin; SI, stratum intermedium; lin, lingual side; lab, labial side.

At P0 (Fig. 1$D_{1,2}$), functional ameloblasts and enamel were covering the labial part of the tooth germ, although only in its anterior portion (Fig. 1D_1). Staining for cleaved caspase-7 was found in the odontoblasts and ameloblasts (Fig. 1E), whereas the neighbor cells of stratum intermedium were caspase-7 negative at this stage (Fig. 1E). In the labial part of the cervical loop, caspase-7 positive cells were detected on the inner portion of the epithelium (Fig. 1F), while the outer epithelium in contact with the peridental mesenchyme remained unstained (Fig. 1C,F).

Later on, at P10 (Fig. 1G), the active form of caspase-7 was detected in the cytoplasm of ameloblasts (Fig. 1H,J) and odontoblasts (Fig. 1I, K) while nuclei in

both cell types were negative. A clear border was observed between the cleaved caspase-7 positive odontoblasts/ameloblasts and the cells of the peridental mesenchyme, next to these ameloblasts, which was negative (Fig. 1L). Caspase-7 positive odontoblasts were detected also in the root-analog of the incisor (data not shown), whereas the stratum intermedium cells remained caspase-7 negative (Fig. 1H). Dental pulp was in general caspase-7 negative at all investigated stages. Few scattered, slightly positive cells were found in the marginal parts beneath the forming odontoblast layer.

Analysis of caspase-7 knock-out incisor phenotype

In the adult caspase-7 deficient mice, the incisors erupted as in wild type littermates (Fig. 2A,D). Despite normal amelogenin expression in the WT and KO (Fig. 2B,E), the histological analysis suggested some differences in the thickness of the hard tissue with less mineralized matrix laid down in the knock-out compared to the wild type (Fig. 2C,F). There were no histologically apparent differences in the stratum intermedium cells of the wild type and deficient mice (Fig. $2G_1$,H_1). Based on PCNA expression in the adult mice, in both, the wt and deficient mice, the lines of differentiated odontoblasts and ameloblasts were negative; few scattered positive cells were detected in the mesenchymal tissue surrounding the labial part of the incisor (Fig. $2G_2$,H_2) and the mesenchyme around the interface of the enamel and enamel-free region of the tooth (Fig. $2G_3$,H_3). MicroCT (Fig. 2I) and statistical analyses (Fig. 2J) confirmed a significant decrease in the amount of mineralized tissue in caspase-7 KO mice.

Discussion

Proteins known for their specific roles in apoptosis have recently been reported as associated with a wide range of non-apoptotic functions such as cell cycle progression, differentiation, self-renewal, metabolism or autophagy (Galluzzi *et al.* 2012). Caspases, as the key proteins/enzymes in the apoptotic machinery (Feinstein-Rotkopf & Arama 2009), are the hottest candidates for such diverse roles.

Dental apoptosis is caspase dependent (Matalova *et al.* 2012a); however, so far only caspase-3 (Matalova *et al.* 2006) and caspase-9 (Setkova *et al.* 2007) have been shown to be essential in molar development as their deficiency leads to absence of apoptotic cells. Caspase-7 was found expressed in the apoptotic regions of the molar tooth signaling centers, the enamel knots, but its absence (knock-out) did not cause any inhibition or prevention of apoptosis (Matalova *et al.* 2012a). Notably, in the molars cleaved caspase-7 was present in non-apoptotic, differentiated cells, particularly odontoblasts and ameloblasts. Despite this expression, deficiency of caspase-7 did not alter molar development in a distinct way (Matalova *et al.* 2012a). Therefore, we turned to the incisor, which is permanently growing in the mouse, and differentiation of cells producing dental hard tissues occurs continuously.

Immunohistochemistry confirmed the presence of cleaved caspase-7 in incisor ameloblasts and odontoblasts, in a similar pattern to the molars (Matalova *et al.* 2012a). In the incisor a difference in expression was shown between the labial and lingual parts of the cervical loop. The epithelial cells in contact with the basement membrane on the lingual side of the incisor were caspase-7 negative. Since these cells do not produce enamel, the absence of caspase-7 cleavage on the lingual side and strong cleavage on the labial side may suggest a role of caspase-7 in formation of hard tissues, unrelated to apoptosis. The expression indicates that caspase-7 may play a role in the fate of dental cells that are producing extracellular matrix and undergoing mineralization. Speculatively, the scattered slightly positive cells in the marginal part of the dental pulp mesenchyme at early stages of development may mirror different fate of pulp located cells as described in molar teeth (Diep *et al.* 2009; Rothova *et al.* 2012).

Furthermore, the caspase-7 deficient incisors displayed a significant decrease in the amount of mineralized matrix. However, the loss of caspase-7 did not cause an absence of enamel in the knock-out but rather delayed mineralization and/or hypomineralization. Similar incomplete impairment in enamel formation was reported in other transgenic animals (Yoshioka *et al.* 2011). There was no obvious difference between localization of amelogenin in the wild type and caspase-7 knock-outs, but it is possible that the levels of such enamel-associated proteins were reduced but this was not quantified in our analysis. MicroCT and histology showed variations in the amount of mineralized matrix. All together, the set of observations reported here supports the hypothesis that active caspase-7 could be involved in the modulation of ameloblast functional differentiation/maturation and not in an on/off regulation. Very complex mechanisms regulate dentinogenesis and amelogenesis (Caton & Tucker 2009; Simmer *et al.* 2010) and the data presented here suggest that caspase-7 may take part in these networks. The hypothesis that caspase-7 is likely to play a role in the differentiation/mineralization of hard tissue/cells is supported by the fact that cleaved caspase-7 can be observed not only in odontoblasts and ameloblasts but also in osteoblasts forming the bone hard tissue (Matalova *et al.* 2012a).

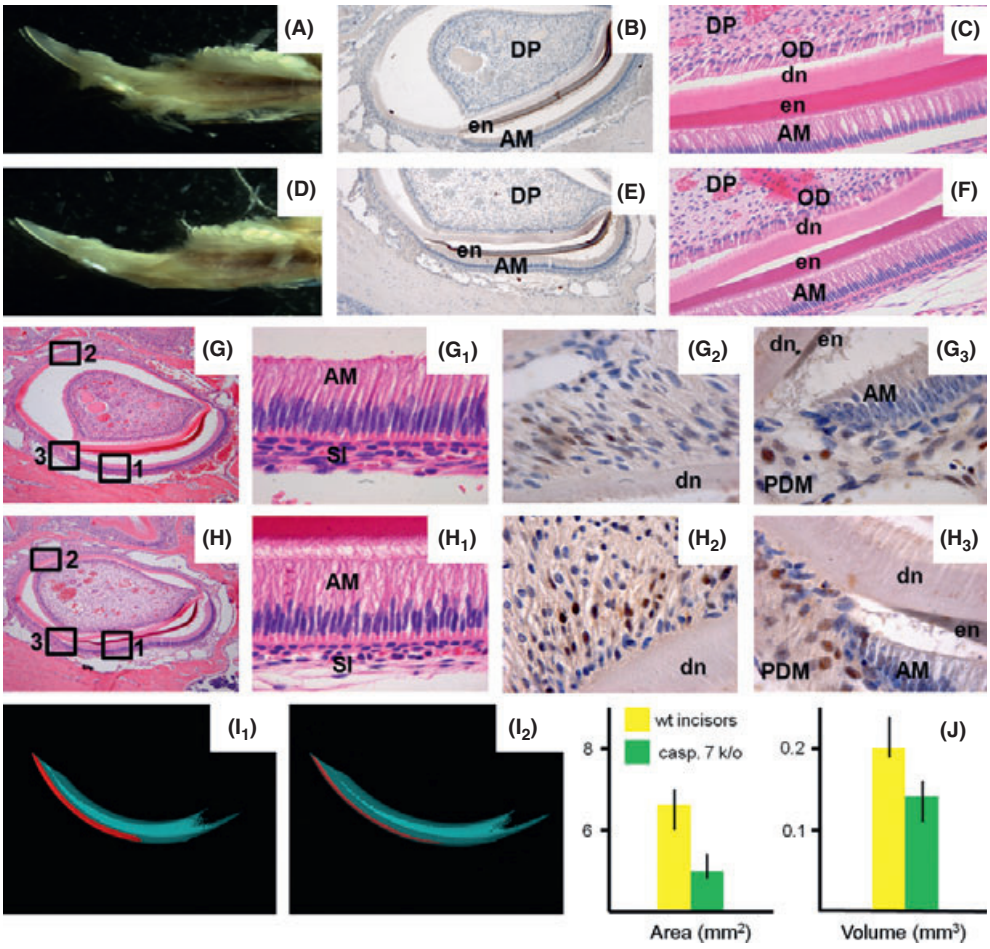

Fig. 2. Analysis of caspase-7 deficient incisors. (A–C) Wild type adult incisor. (D–F) Caspase-7 knock-out mouse. (A) Macroscopic view of fully erupted adult wild type incisor. Scale bar = 3 mm. (B) Immunohistochemistry of amelogenin in the adult wild type incisor. Scale bar = 100 μm. (C) Detailed view at dentin (and odontoblasts) and enamel (and ameloblasts) structure and deposition in the adult wild type mouse. Scale bar = 50 μm. (D) Macroscopic view of fully erupted adult incisor of the caspase-7 deficient mouse. Scale bar = 3 mm. (E) Immunohistochemistry of amelogenin in the adult type incisor of the caspase-7 deficient mouse. Scale bar = 100 μm. (F) Detailed view at dentin (and odontoblasts) and enamel (and ameloblasts) structure and deposition in the adult caspase-7 deficient mouse. Scale bar = 50 μm. (G) Hematoxylin/Eosin stained sagittal section of the wild type incisor, indicating regions magnified in $G_{1,2,3}$. (G_1) Detailed view at the stratum intermedium cells of the wild type incisor. (G_2) Proliferation (PCNA staining) in the mesenchymal cells surrounding the labial part of the wild type incisor. (G_3) Proliferation (PCNA staining) in the mesenchymal cells surrounding the enamel and enamel-free region interface of the wild type incisor. (H) Hematoxylin/Eosin stained sagittal section of the caspase-7 deficient incisor, indicating regions magnified in $H_{1,2,3}$. (H_1) Detailed view at the stratum intermedium cells of the caspase-7 deficient incisor. (H_2) Proliferation (PCNA staining) in the mesenchymal cells surrounding the labial part of the caspase-7 deficient incisor. (H_3) Proliferation (PCNA staining) in the mesenchymal cells surrounding the enamel and enamel-free region interface of the caspase-7 deficient incisor. (I) MicroCT of the adult incisor in the wild type (I_1) and caspase-7 deficient mice (I_2) confirmed decreased mineralization in the caspase-7 incisors. Scale bar = 2 mm. (J) Statistical evaluation of the microCT results. Both, the enamel area and volume display significantly lower value in the knock-out than in the wild type. AM, ameloblasts; dn, dentin; DP, dental papilla; en, enamel; OD, odontoblasts; pAM, preameloblasts; PDM, peridental mesenchyme; SI, stratum intermedium.

Although it has been shown that some caspases, such as caspase-3, are required for differentiation of a variety of cell types (Larsen *et al.* 2010), the mechanism allowing caspases to influence cell-fate decision and regulate differentiation, survival or proliferation remains unclear.

Subcellular localization of cleaved caspase-7 seems to correspond with its particular function. During apoptosis, activated caspases becomes translocated into the nucleus (Lamkanfi & Kanneganti 2010), whereas cleaved caspase-7 in non-apoptotic areas of the incisor was found exclusively in the cytoplasm.

This observation is supported by the finding that in developing molars caspase-3 is localized in the nucleus of apoptotic cells (Matalova *et al.* 2006); while it is localized to the cytoplasm of non-apoptotic epithelial cells (Kumamoto *et al.* 2001). The cytoplasmic localization of caspase-7 appears similar in molars and incisors in non-apoptotic regions of the tooth (Matalova *et al.* 2012b), indicating that the localization of cleaved protein regulates its function.

One hypothesis suggesting the possible mechanisms of caspase-7 function based on its localization within the tooth germ would be engagement in differentiation/maturation of cells by cleavage of proteins typical for the undifferentiated status of cells. In such cases, the non-apoptotic mechanism of caspase function may include the depletion of stem cell markers, OCT4 and NANOG, as demonstrated in cancer cells after drug treatment (Musch *et al.* 2010). In the study, caspase-3 cleaved NANOG, whereas caspase-7 was able to degrade both of these markers. Oct3/4 was reported to be strongly expressed in the nucleus of the incisor cervical loop cells starting at E18, persisting up to late postnatal stages (Li *et al.* 2011). As there is no universal marker of the stem cell niche in the incisors so far, Oct3/4 is one of the molecules expected in dental stem cells (Li *et al.* 2011). Caspase-7 was located in the inner part of the labial cervical loop, while the outer region was caspase-7 negative. This caspase-7 negative domain correlates with the region of the cervical loop where Oct3/4 is present and to the putative location of stem cells as indicated by Sox2 (Li *et al*. 2011). In contrast to the cervical loop, Oct3/4 is expressed in the cytoplasm in ameloblasts and odontoblasts (Li *et al.* 2011) similar to the active form of caspase-7. These findings could suggest some putative participation of caspase-7 in the cleavage of Oct4 before/during cell differentiation. Depletion of stem cell factors was shown to be mediated by cleaved caspases and OCT4 is a direct *in vitro* target of active caspase-7 (Musch *et al.* 2010).

In this context, the expression of Oct4 was previously reported in relation to different cell populations during tooth root formation (Nakagawa *et al.* 2012). A similar difference was detected between the regions we show that are caspase-7 positive (ameloblasts) and negative (stratum intermedium and peridental mesenchyme) in the incisors. Therefore, the cleaved form of caspase-7, connected with Oct4, could be a further marker to distinguish these different cell populations.

Alternatively, caspase-7 might participate in the activation of nuclear factor-κB (NF-κB). A link between these pathways has been demonstrated by the effect of caspase-3 on osteoclasts (Szymczyk *et al.* 2006) or lens (Basu *et al.* 2012) differentiation. A similar role in teeth is supported by the importance of tumor necrosis factor (TNF) signaling in development and evolution of tooth number, size and shape (Ohazama & Sharpe 2004). Moreover, Fas receptor, as a member of the TNF family, was suggested to have a modulatory function in dental apoptosis (Matalova *et al.* 2005) and might play a role in a switch in cell fate, as has been reported in apoptotic versus non-apoptotic decisions in other systems (Lavrik & Krammer 2012). Recent data indicate that non-apoptotic functions of caspases involve proteolysis exerted by their catalytic domains as well as non-proteolytic functions exerted by their prodomains (Lamkanfi *et al.* 2007).

Confirmation of such hypotheses, as well as the final matching of caspase-7 into other molecular networks governing differentiation, maturation and function of hard tissue cells, is an important area for further analysis.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Basu, S., Rajakaruna, S. & Menko, A. S. 2012. Insulin-like growth factor receptor-1 and nuclear factor kappa B are crucial survival signals that regulate caspase-3-mediated lens epithelial cell differentiation initiation. *J. Biol. Chem.* **287**, 8384–8397.

Caton, J. & Tucker, A. S. 2009. Current knowledge of tooth development, patterning and mineralization of the murine dentition. *J. Anat.* **214**, 502–515.

Diep, L., Matalova, E., Mitsiadis, T. A. & Tucker, A. S. 2009. Contribution of the tooth bud mesenchyme to alveolar bone. *J. Exp. Zool. B Mol. Dev. Evol.* **312B**, 510–517.

Duprez, L., Takahashi, N., Van Hauwermeiren, F., Vandendriessche, B., Goossens, V., Vanden Berghe, T., Declercq, W., Libert, C., Cauwels, A. & Vandenabeele, P. 2011. RIP kinase-dependent necrosis drives lethal systemic inflammatory response syndrome. *Immunity* **35**, 908–918.

Feinstein-Rotkopf, Y. & Arama, E. 2009. Can't live without them, can live with them, roles of caspases during vital cellular processes. *Apoptosis* **14**, 980–995.

Fujita, J., Crane, A. M., Souza, M. K., Dejosez, M., Kyba, M., Flavell, R. A., Thomson, J. A. & Zwaka, J. 2008. Caspase

activity mediates the differentiation of embryonic stem cells. *Cell Stem Cell* **2**, 595–601.

Galluzzi, L., Kepp, O., Trojel-Hansen, C. & Kroemer, G. 2012. Non-apoptotic functions of apoptosis-regulatory proteins. *EMBO Rep.* **13**, 322–330.

Harada, H., Kettunen, P., Jung, H. S., Mustonen, T., Wang, Y. A. & Thesleff, I. 1999. Localization of putative stem cells in dental epithelium and their association with notch and FGF signaling. *J. Cell Biol.* **147**, 105–120.

Kieffer-Combeau, S., Meyer, J. M. & Lesot, H. 2001. Cell-matrix interactions and cell-cell junctions during epithelial histo-morphogenesis in the developing mouse incisor. *Int. J. Dev. Biol.* **45**, 733–742.

Kumamoto, H., Kimi, K. & Ooya, K. 2001. Immunohistochemical analysis of apoptosis-related factors Fas, Fas ligand, caspase-3 and single stranded DNA. in ameloblastomas. *J. Oral Pathol. Med.* **30**, 596–602.

Kuranaga, E. 2012. Beyond apoptosis, caspase regulatory mechanisms and functions *in vivo*. *Genes Cells* **17**, 83–97.

Lamkanfi, M., Festjens, N., Declercq, W., Vanden Berghe, T. & Vandenabeele, P. 2007. Caspases in cell survival, proliferation and differentiation. *Cell Death Differ.* **14**, 44–55.

Lamkanfi, M. & Kanneganti, T.-D. 2010. Caspase-7, A protease involved in apoptosis and inflammation. *Int. J. Biochem. Cell Biol.* **42**, 21–24.

Lapthanasupkul, P., Feng, J. F., Mantesso, A., Takada-Horisawa, Y., Vidal, M., Koseki, H., Wang, L. L., An, Z. W., Miletich, I. & Sharpe, P. T. 2012. Ring1a/b polycomb proteins regulate the mesenchymal stem cell niche in continuously growing incisors. *Dev. Biol.* **367**, 140–153.

Larsen, B. D., Rampalli, S., Burns, L. E., Brunette, S., Dilworth, F. J. & Megeney, L. A. 2010. Caspase 3/caspase-activated DNase promote cell differentiation by inducing DNA strand breaks. *Proc. Natl Acad. Sci. USA* **9**, 4230–4235.

Lavrik, I. L. & Krammer, P. 2012. Regulation of CD95/Fas signaling at the DISC. *Cell Death Differ.* **19**, 36–41.

Li, L., Kwon, H. J., Harada, H., Ohshima, H., Cho, S. W. & Jung, H. S. 2011. Expression patterns of ABCG2, Bmi-1, Oct 3/4, and Yap in the developing mouse incisor. *Gene Expr. Patterns* **11**, 163–170.

Martinon, F. & Tschopp, J. 2007. Inflammatory caspases and inflammasomes, master switches of inflammation. *Cell Death Differ.* **14**, 10–22.

Matalova, E., Sharpe, P. T., Lakhani, S. A., Roth, K. A., Flavell, R. A., Setkova, J., Misek, I. & Tucker, A. S. 2006. Molar tooth development in caspase-3 deficient mice. *Int. J. Dev. Biol.* **50**, 491–497.

Matalova, E., Svandova, E. & Tucker, A. S. 2012a. Apoptotic signaling in mouse odontogenesis. *OMICS* **16**, 60–70.

Matalova, E., Tucker, A. S. & Misek, I. 2005. Apoptosis-related factors Fas receptor, Fas ligand, FADD. in early tooth development of the field vole Microtus agrestis. *Arch. Oral Biol.* **50**, 165–169.

Matalova, E., Tucker, A. S. & Sharpe, P. T. 2004. Death in the life of a tooth. *J. Dent. Res.* **83**, 11–16.

Matalova, E., Vanden Berghe, T., Svandova, E., Vandenabeele, P., Healy, C., Sharpe, P. T. & Tucker, A. S. 2012b. Caspase-7 in molar tooth development. *Arch. Oral Biol.* **57**, 1474–1481.

Miura, M., Chen, X. D., Allen, M. R., Bi, Y. M., Gronthos, S., Seo, B. M., Lakhani, S., Flavell, R. A., Feng, X. H., Robey, P. G., Young, M. & Shi, S. T. 2004. A crucial role of caspase-3 in osteogenic differentiation of bone marrow stromal stem cells. *J. Clin. Invest.* **114**, 1704–1713.

Musch, T., Oz, Y., Lyko, F. & Breiling, A. 2010. Nucleoside drug induce cellular differentiation by caspase-dependent degradation of stem cell factors. *PLoS One* **5**, 1–15.

Nakagawa, E., Zhang, L., Shin, J. Q., Kim, E. J., Cho, S. W., Ohshima, H., Chen, Z. & Jung, H. S. 2012. The novel expression of Oct3/4 and Bmi1 in the root development of mouse molars. *Cell Tissue Res.* **357**, 479–484.

Ohazama, A. & Sharpe, P. T. 2004. TNF signalling in tooth development. *Curr. Opin. Genet. Dev.* **14**, 513–519.

Peterkova, R., Peterka, M. & Lesot, H. 2003. The developing mouse dentition - A new tool for apoptosis study. *Ann. N. Y. Acad. Sci.* **1010**, 453–466.

Rothova, M., Peterkova, R. & Tucker, A. S. 2012. Fate map of the dental mesenchyme: dynamic development of the dental papilla and follicle. *Dev. Biol.* **365**, 244–254.

Setkova, J., Matalova, E., Sharpe, P. T., Misek, I. & Tucker, A. S. 2007. Primary enamel knot cell death in Apaf-1 and caspase-9 deficient mice. *Arch. Oral Biol.* **52**, 15–19.

Simmer, J., Papagerakis, P., Smith, C. E., Fisher, D. C., Rountrey, A. N., Zheng, L. & Hu, J. C. C. 2010. Regulation of dental enamel shape and hardness. *J. Dent. Res.* **89**, 1024–1038.

Yoshioka, H., Yoshiko, Y., Minamizaki, T., Suzuki, S., Koma, Y., Nobukiyo, A., Sotomaru, Y., Suzuki, A., Itoh, M. & Maeda, N. 2011. Incisor enamel formation is impaired in transgenic rats overexpressing the type III NaPi transporter Slc20a1. *Calcif. Tissue Int.* **89**, 192–202.

Szymczyk, K. H., Freeman, T. A., Adams, C. S., Srinivas, V. & Steinbeck, M. J. 2006. Active caspase-3 is required for osteoclast differentiation. *J. Cell Physiol.* **209**, 836–844.