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CD 95 mediated apoptosis in embryogenesis: implication in tooth development

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Structured abstract

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Introduction – Understanding of apoptotic mechanisms involved in tissue shaping is of particular interest because of possible targeted modulation of the development of organ structures such as teeth. Research of CD 95 mediated apoptosis has been focused particularly on cell death in the immune system and related disorders. However, CD 95 mediated apoptosis is also involved in embryogenesis of many organs as the kidney, the lung, the intestine and tissue networks such as the nervous system.

Design – Narrative review.

Results – This review briefly summarizes the current knowledge of CD 95 mediated apoptosis in embryogenesis with possible implication in tooth development. CD 95 receptor and CD 95 ligand are found at early stages of tooth development. The data suggest some positive correlations with dental apoptosis distribution, particularly in the primary enamel knot where apoptosis occurs during elimination of this structure. CD 95 deficient (lpr) adult mouse tooth phenotype, however, did not show any alterations in final tooth pattern and morphology.

Conclusion – To date studies of apoptotic machinery during tooth development show spatial localization of many of the components together with precise and localized timing of cell death. There is still much to be learned about the regulation and importance of apoptosis in tooth development. Nevertheless, the involvement of apoptotic regulatory mechanisms interplaying with other molecules participates to the cellular cross-talk in developing tissues, which opens possible targeted modulations as suggested, e.g. for future molecular dentistry.

Key words: Fas; embryonic development; odontogenesis; review

Apoptosis in embryogenesis and odontogenesis

Apoptosis as an evolutionally conserved phenomenon of programmed cell destruction has been involved in a range of physiological processes from embryogenesis to everyday homeostasis maintenance. During development, apoptosis participates particularly in structural shaping and elimination of unwanted cells, regulation of cell number within tissues, elimination of incorrectly localized or non-functional cells. Identification of a wide spectrum of pro-apoptotic and anti-apoptotic genes and their interactions has contributed to knowledge of cellular mechanisms driving apoptotic cell death and to understanding the morphogenetic events in embryogenesis.

Following pioneering studies in *C. elegans*, the current picture of developmental events shows many central roles of apoptosis in a number of developmental processes in mammals. Maturation of the immune and neuronal systems undoubtly involves apoptotic cell death (1,2). Apoptosis has also been demonstrated as a sculptor of development (3), particularly in branching morphogenesis essential for proper development of several organ tissues as the lung, the kidney and the mammary and salivary glands (4,5). Apoptosis is also evident in morphogenesis of craniofacial structures such as the inner ear (6), palatine rugae (7), alveolar bone (8), temporomandibular joint (9) and teeth.

Tooth development (odontogenesis), is based on reciprocal communication between epithelium and underlying mesenchyme, and involves activation of specific genes (10) including odontogenic homeoboxes (11). This molecular patterning is followed by the first histological signs of developing teeth. Apoptosis is not only balancing cell proliferation in the tooth germ, but also seems to have an impact on tooth morphogenesis. On the basis of temporospatial distribution of apoptotic cells (12,13), dental apoptosis is suggested to have passive and active roles in tooth development. However, functional confirmation of morphogenetic roles of dental apoptosis and the exact evidence on mechanisms employed are still missing (14,15).

CD 95 mediated apoptosis in embryogenesis and odontogenesis

Apoptosis undoubtedly takes part in embryogenic tissue remodelling (16). However, not much is known about the exact mechanisms involved. Members of the tumour necrosis factor (TNF) family participate in many cellular events such as organogenesis, immune reaction and also apoptosis. TNF receptors are activated after binding of a corresponding ligand and can trigger different intracellular signalling pathways. However, they can act also as decoy receptors. The most common machinery activated by the receptor–ligand complex occurs through an adaptor domain, such as a death domain in an apoptotic cascade or in a TRAF transduction pathway involving Jun kinases and NF-kappa B.

TNF/NK-kappa B signalling has been shown to be engaged in tooth cusp morphogenesis in molar teeth (17,18). Eda, EdaR (receptor) and EdarADD (associated death domain) have been studied using spontaneous mouse mutations Tabby (19), Downless (20) and Crinkled (21). Eda/Edar interactions were demonstrated to regulate enamel knot formation in tooth morphogenesis (22).

CD 95 (Fas⁄Apo-) represents another candidate receptor engaged in dental apoptosis. CD 95 receptor and ligand mRNA were found in mouse embryos as well as adult mice, and were co-expressed particularly in tissues characterized by apoptotic cell turnover (23). CD 95 participates in involution and remodelling of the reproductive system (24,25), motoneuron networks (26,27), bone formation (28–30), eye angiogenesis (31) and has also been found in the craniofacial region (32). In teeth, CD 95 was consistently detected during the secretion, transition and maturation of ameloblasts (33). Key components of the CD 95 initiating complex were detected also in early development of molar tooth germs, particularly in the primary enamel knot (34).

The primary enamel knot is characterized as a transitory structure with a specific arrangement of cells and accumulation of apoptotic activity (12). Specific expression of several signalling molecules such as Shh, BMP-2, 4, 7, FGF-4 can be found in the enamel knot cells (35,36) and therefore enamel knots have been considered to act as signalling centres for tooth morphogenesis.

CD 95 belongs to membrane I receptors (with intracellular localization of C terminal domain) with constitutional expression in lymphoid tissue (37). However, CD 95 is found on the surface of most cells (38). Alternative splicing can produce a soluble form of the receptor, without the transmembrane domain but still with the ligand-binding affinity (39). Both, extracellular and intracellular termini of the CD 95 receptor were located in the areas of primary enamel knot where apoptosis occurred as confirmed by morphological and biochemical criteria (34).

The best understood physiological functions of CD 95 system are regulations within the immune system, also confirmed by mutant analyses of CD 95 deficiency– lpr phenotype (40) and CD 95 ligand deficiency – glc phenotype (41), both autosomal recessive. The abnormal phenotypes of lymphoadenopathy and autoimmune disorders are caused by impairment in apoptosis induction. Adult tooth phenotype of lpr mice was studied to reveal morphological alterations caused by CD 95 deficiency. However, has not revealed any impact of CD 95 knock-out on the final crown tooth morphology (unpublished data). This finding suggests some possible hypotheses. CD 95 signalling may not be involved in dental apoptosis but other pathways (42), or CD 95 may rather be enhancing and promoting dental apoptosis than triggering this process, or CD 95 molecules can be inhibited by anti-apoptotic members such as Bcl-2 (43,32) at protein–protein level, or dental apoptosis in general simply may not have any impact on final crown morphogenesis. The latter explanation is supported by the only functional study using caspase (and thus apoptosis) inhibition in the primary enamel knot (44) where no alterations in the tooth crown morphogenesis were observed after 2 days caspase inhibition in explant culture system. Nevertheless, apoptosis has still been considered as a sculptor of development (3) and may also have this role in tooth formation.

Other related molecules in embryogenic and dental apoptosis

Apoptosis can be communicated by several extra – and intracellular triggering of three general pathways leading to cell self-destruction: receptor mediated activation, mitochondria breakdown and alterations in endoplasmic reticulum (45). In the intracellular cell death machinery, caspases are considered to be the main mediators (46). Consequent signalling following triggering involves cascade reactions based on activation of different molecules and/or switching on/off specific gene expression.

The mitochondria mediated apoptotic programme can be switched by some kinds of cellular stress. This signalling converges at the mitochondrial level and initiates opening of transition pores followed by release of proapoptotic factors as cytochrome-c (47,48), Smad/Diablo (49), AIF (50), endonucleases (51). Members of Bcl-2 family involving both, pro-apoptotic (e.g. Bax, Bim, and Bid) and anti-apoptotic (e.g. Bcl-2 and Mcl-1) molecules (52), act as regulators of mitochondrial apoptotic alterations.

Activation of specific membrane surface death receptors, by binding their corresponding ligand molecules, starts apoptotic pathways in the cells (Fig. 1). TNF receptor family involving CD 95 (Fas and Apo-1) receptor along with others such as TNFR1 (p55 and CD120a), DR3 (Apo-3, WSL-1, TRAMP, and LARD), DR4 (Apo-2 and TRAIL-R1), DR5 (TRAIL-R2, TRICK 2, and KILLER) belong to widely studied molecules in apoptotic signal transduction (53). These apoptotic receptors display a homology in the extracellular cysteine rich subdomain and also in the intracellular part, the so-called death domain (DD) (54). DD enables association of receptor with cytosolic adaptor molecules such as FADD and TRADD (55,56), mediating signal transduction to other signalling components (56). The N-terminus of FADD death effector domain, recruites procaspase-8 followed by formation of death-inducing signalling complex (DISC). N-terminus of procaspase-8 binds and activates other downstream caspases such as caspase-3, -4 or -7 (57,58). The caspase execution can proceed in two different pathways, direct cleavage (59) or an indirect effect communicated by release of mitochondrial factors (60).

Interplay of BMP-4 inductive signals, FGF molecules and homeobox genes such as Msx-1 and Msx-2 (61–64) as well as other signalling pathways such as Shh and Wnt (64–65) have been considered as modulators of dental apoptosis. Specific molecular events may also serve as a driving force for death induced by trophic deprivation. However, primary enamel knot cells undergoing apoptosis do not have receptors for growth factors such as FGF-4 even when they secrete this potent mitogen (66). In the absence of trophic support,

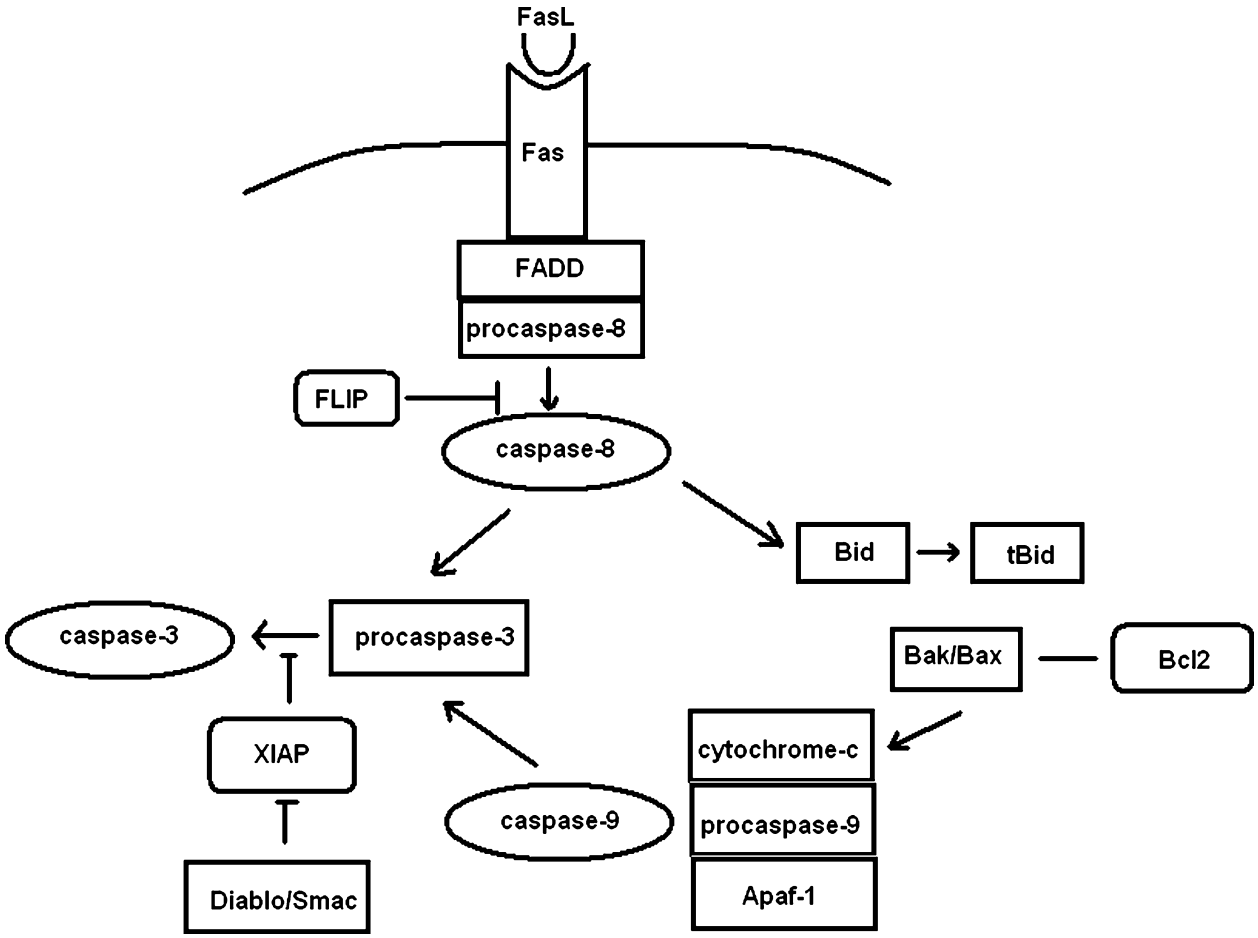

Fig. 1. Schematic drawing of the basic apoptotic pathways induced by CD95 receptor – CD95 ligand interactions. The apical caspase-8 is activated in death inducing signalling complex (DISC) – activated CD95 receptor, FADD, procaspase-8. Caspase-8 either directly cleaves procaspase-3 or Bid which starts mitochondria mediated pathway via apoptosome formation – cytochrome-c, procaspase-9, Apaf-1 followed by caspase-9 activation and procaspase-3 cleavage. The central caspase-3 executes further cleavage of substrates.

CD 95 becomes sufficiently activated and binding of CD 95 ligand could promote the apoptotic death in the paracrine fashion. This could support more rapid liquidation of apoptotic cells by caspase mediated cleavage and thus more effective elimination by apoptotic debris in the developing tooth organ where professional phagocytes are still absent (67). Moreover, both receptor mediated and intrinsic apoptotic machinery can be switched in individual cells within the population and depend on different signals (68). Further functional experiments would be required to determine the role of CD 95 system in odontogenesis.

Conclusion

To date studies of apoptotic machinery during tooth development show spatial localization of many of the components together with precise and localized timing of cell death. Mice with null mutations in several of the key molecules, however, do not exhibit major tooth phenotypes. There is still much to be learned about the regulation and importance of apoptosis in tooth development. Nevertheless, the involvement of apoptotic regulatory mechanisms interplaying with other molecules participates to the cellular cross-talk in developing tissues, which opens possible targeted modulations as suggested, e.g. for future molecular dentistry (69).

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References

1. Opferman JT, Korsmeyer SJ. Apoptosis in the development and maintenance in the immune system. *Semin Immunol* 2003; **4**:410–5.
2. Buss RR, Oppenheimer RW. Role of programmed cell death in normal neuronal development and function. *Anat Sci Int* 2004;**79**:191–7.
3. Doseff AI. Apoptosis: the sculptor of development. *Stem Cells Dev* 2004;**13**:473–83.
4. Humphreys RC, Krajewska M, Krnacik S, Jaeger R, Weiher H, Krajewski S et al. Apoptosis in the terminal endbud of the murine mammary gland: a mechanism of ductal morphogenesis. *Development* 1996;**122**:227–46.
5. Piscione TD, Rosenblum ND. The molecular control of renal branching morhogenesis. *Differentiation* 2002;**70**:227–46.
6. Cecconi F, Roth KA, Dolgov O, Munarriz E, Anokhin K, Gruss P et al. Apaf1-dependent programmed cell death is required for inner ear morphogenesis and growth. *Development* 2004;**131**:2125–35.
7. Amasaki H, Ogawa M, Nagasao J, Mutoh K, Ichihara N, Asari M. Distribution of apoptotic cells and apoptosis – related molecules in the developing murine palatine rugae. *J Vet Med Sci* 2002;**64**:1103–6.
8. Hamaya M, Mizoguchi I, Sakakura Y, Yajima T, Abiko Y. Cell death of osteocytes occurs in rat alveolar bone during experimental tooth movement. *Calcif Tissue Int* 2002;**70**:117–26.
9. Matsuda S, Mishima K, Yoshimura Y, Hatta T, Otani H. Apoptosis in development of the temporospatial joint. *Anat Embryol* 1997;**196**:383–91.
10. Cobourne MT, Sharpe PT. Tooth and jaw: molecular mechanisms of patterning in the first branchial arch. *Arch Oral Biol* 2003;**48**:1–14.
11. Sharpe PT. Homeobox genes and orofacial development. *Connect Tissue Res* 1995;**32**:17–25.
12. Lesot H, Vonesch JL, Peterka M, Turečková J, Peterková R, Ruch JV. Mouse molar morphogenesis revised by three-dimensional reconstruction. II. Spatial distribution of mitoses and apoptosis in cap to bell stages first and second upper molar teeth. *Int J Dev Biol* 1996;**40**:1017–31.
13. Matalova E, Witter K, Misek I. Apoptosis distribution in the first molar tooth germ of the field vole (Microtus agrestis). *Tissue Cell* 2004b;**36**:361–7.
14. Peterkova R, Peterka M, Lesot H. The developing mouse dentition: a new tool for apoptosis study. *Ann N Y Acad Sci* 2003;**1010**:453–66.
15. Matalova E, Tucker AS, Sharpe PT: Death in the life of a tooth. *J Dent Res* 2004a;**83**:11–16.
16. Meier P, Finch A, Evan G. Apoptosis in development. *Nature* 2000;**407**:796–801.
17. Ohazama A, Sharpe PT. TNF signalling in tooth development. *Curr Opin Genet Dev* 2004a;**14**:513–9.
18. Tucker AS, Headon DJ, Courtney JM, Overbeek P, Sharpe PT. The activation level of the TNF family receptor, Edar, determines cusp number and tooth number during tooth development. *Dev Biol* 2004;**268**:185–94.
19. Kere J, Srivastava AK, Montonen O, Zonana J, Thomas N, Ferguson B et al. X-linked anhidrotic (hypohidrotic) ectodermal dysplasia is caused by mutation in a novel transmembrane protein. *Nat Genet* 1996;**13**:409–16.
20. Headon DJ, Overbeek PA. Involvement of a novel TNF receptor homologue in hair follicle induction. *Nat Genet* 1999;**22**:370–4.
21. Headon DJ, Emmal SA, Ferguson BM, Tucker AS, Justice MJ, Sharpe PT et al. Gene defect in ectodermal dysplasia implicates a death domain adapter in development. *Nature* 2001;**414**:913–5.
22. Tucker AS, Headon DJ, Schneider P, Ferguson BM, Overbeek P, Tschopp et al. Edar/Eda interactions regulate enamel knot formation in tooth morphogenesis. *Development* 2000;**127**:4691–700.
23. French LE, Hahne M, Viard I, Radlgruber G, Zanone R, Becker K et al. Fas and Fas ligand in embryos and adult mice: ligand expression in several immune privileged tissues and coexpression in adult tissues characterized by apoptotic cell turnover. *J Cell Biol* 1996;**133**:335–43.
24. Suzuki A, Matsuzawa A, Iguchi T. Down regulation of Bcl-2 is the first step on Fas-mediated apoptosis of male reproductive tract. *Oncogene* 1996;**13**:31–37.
25. Mor G, Straszewski S, Kamsteeg M. Role of the Fas/Fas ligand system in female reproductive organs: survival and apoptosis. *Biochem Pharmacol* 2002;**64**:1305–15.
26. Raoul C, Henderson CE, Pettmann B. Programmed cell death of embryonic motoneurons triggered through the Fas death receptor. *J Cell Biol* 1999;**147**:1049–62.
27. Lautrette C, Giraud S, Vermot-Desroches C, Preud'Homme JL, Jauberteau MO. Expression of a functional Fas death receptor by human foetal motoneurons. *Neuroscience* 2003;**119**:377–85.
28. Mori S, Nose M, Chiba M, Narita K, Kumagai M, Kosaka H et al. Enhancement of ectopic bone formation in mice with a deficit in Fas-mediated apoptosis. *Pathol Int* 1997;**47**:112–6.
29. Katavic V, Grcevic D, Lukic IK, Vucenik V, Kovacic N, Kalajzic I et al. Non-functional Fas ligand increases the formation of cartilage early in the enchondral bone induction by rhBMP-2. *Life Sci* 2003;**74**:13–28.
30. Wu X, McKenna MA, Feng X, Nagy TR, McDonald JM. Osteoclast apoptosis: the role of Fas in vivo and in vitro. *Endocrinology* 2003;**144**:5545–55.
31. Kaplan HJ, Leibole MA, Tezel T, Ferguson TA. Fas ligand (CD 95 ligand) controls angiogenesis beneath the retina. *Nat Med* 1999;**5**:292–7.
32. Hatakeyama S, Tomichi N, Ohara-Nemoto Z, Satoh M. The immunohistochemical localization of Fas and Fas ligand in jaw bone and tooth germ of human fetuses. *Calcif Tissue Int* 2000;**66**:330–7.
33. Nishikawa S. Colchicine-induced apoptosis and anti-Fas localization in rat-incisor ameloblasts. *Anat Sci Int* 2002;**77**:175–81.
34. Matalova E, Tucker AS, Misek I. Apoptosis-related factors (Fas receptor, Fas ligand, FADD) in early tooth development of the field vole *(Microtus agrestis)*. *Arch Oral Biol* 2005;**50**:165–9.
35. Jernvall J, Aberg T, Kettunen P, Keranen S, Thesleff I. The life history of an embryonic signalling center: BMP-4 induces p-21 and is associated with apoptosis in the mouse tooth enamel knot. *Development* 1998;**125**:161–9.
36. Vaahtokari A, Aberg T, Thesleff I. Apoptosis in developing tooth: association with an embryonic signalling center and suppression of EGF and FGF-4. *Development* 1996;**122**:121–9.
37. Green DR, Ferguson TA. The role of Fas ligand in immune privilege. *Nat Rev Mol Cell Biol* 2001;**2**:917–24.
38. Nagata S. Fas ligand induced apoptosis. *Ann Rev Gen* 1999;**33**: 29–55.
39. Cascino I, Fiucci G, Papoff G, Ruberti G. Three functional soluble forms of the human apoptosis inducing Fas molecule are produced by alternative splicing. *J Immunol* 1995;**154**:2706–13.

40. Watanabe-Fukunaga R, Brannan CI, Copeland NG, Jenkins NA, Nagata S. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature* 1992;**356**:314–7.
41. Takahashi T, Tanaka M. Brannan CI, Jenkins NA, Copeland NG, Suda T et al. Generalized lymphoproliferative disease in mice, caused by point mutation in the Fas ligand. *Cell* 1994;**76**:969–76.
42. Budd RC. Death receptors couple to both cell proliferation and apoptosis. *J Clin Invest* 2002;**109**:437–41.
43. Scaffidi C, Fulda S, Srinivasan A, Friesen C, Li F, Tomaselli KJ et al. Two CD95 (APO-1/Fas) signalling pathways. *EMBO J* 1998;**17**:1675–87.
44. Coin R, Kieffer S, Lesot H, Vonesch J-L, Ruch J-V. Inhibition of apoptosis in the primary enamel knot does not affect specific tooth crown morphogenesis in the mouse. *Int J Dev Biol* 2000;**44**:389–96.
45. Kidd VJ, Lahti JM, Teitz T. Proteolytic regulation of apoptosis. *Semin Cell Dev Biol* 2000;**11**:191–201.
46. Chang HY, Yang X. Proteases cell suicide: functions and regulation of caspases. *Microbiol Mol Biol Res* 2000;**64**:821–46.
47. Zou H, Henzel WJ, Liu X, Lutshg A, Wang X. Apaf-1, a human protein homologous to *C. elegans* CED-4, participates in cytochrome c dependent activation of caspase-3. *Cell* 1997;**90**:405–13.
48. Li P, Nijhavan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES et al. Cytochrome c and dATP dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* 1997;**91**:479–89.
49. Du C, Fang M, Li Y, Li L, Wang X. Smac, a mitochondrial protein that promotes cytochrome c dependent caspase activation by eliminating IAP inhibition. *Cell* 2000;**102**:33–42.
50. Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM et al. Molecular characterization of mitochondrial apoptosis-inducing factor. *Nature* 1999;**397**:441–6.
51. Li P, Luo X, Wang X. Endonuclease G is an apoptotic DNase when eleased from mitochondria. *Nature* 2001;**412**:95–99.
52. Antonsson B, Martinou JC. The Bcl-2 protein family. *Exp Cell Res* 2000;**256**:50–57.
53. Schmitz I, Kirchhoff S, Krammer PH. Regulation of death receptor mediated apoptosis pathways. *Int J Biochem Cell Biol* 2000;**32**:1123–36.
54. Muzio M. Signalling by proteolysis: death receptors induce apoptosis. *Int J Clin Lab Res* 1998;**28**:141–7.
55. Chinnaiyan AM, O'Rourke K, Tewari M, Dixit VM. FADD, a novel death domain containing protein, interacts with the death domain of Fas and initiates apoptosis. *Cell* 1995;**81**: 505–12.
56. Hsu H, Xiong J, Goeddel DV. The TNF receptor 1 associated protein TRADD signals cell death and NF-kappaB activation. *Cell* 1995;**81**:495–504.
57. Boldin MP, Goncharov TM, Goltsev YV, Wallach D. Involvement of MACH, a novel MORTI/FADD interacting protease, in Fas/APO-1 and TNF receptor-induced cell death. *Cell* 1996;**85**:803–15.
58. Muzio M, Chinnaiyan AM, Kischkel FC, O'Rourke K, Shevchenko A, Ni J et al. FLICE, a novel FADD-homologous ICE/CED-3 like protease, is required to the CD95 (Fas/Apo1) death inducing signal complex. *Cell* 1996;**85**:817–27.
59. Srinivasula SM, Ahmad M, Fernandes-Alnemri T, Litwack G, Alnemri ES. Molecular ordering of the Fas-apoptotic pathway: the Fas/APO-1 protease Mch5 is a CrmA inhibitable protease that activates multiple CED-3/ICE-like cysteine proteases. *Proc Natl Acd Sci USA* 1996;**93**:14486–91.
60. Kuwana T, Smith JJ, Muzio M, Dixit V, Newmeyer DD, Kornbluth S. Apoptosis induction by caspase-8 is amplified through the mitochondrial release of cytochrome c. *J Biol Chem* 1998;**273**:16589–94.
61. Jernvall J, Kettunen P, Karavanova I, Martin LB, Thesleff I. Evidence for the role of the enamel knot as a control center in mammalian tooth cusp formation – nondividing cells express growth stimulating FGF-4 gene. *Int J Development Biol* 1994;**38**:463–9.
62. Bei M, Maas R. FGFs and BMP4 induce both Msx1-independent and Msx1-dependent signalling pathways in early tooth development. *Development* 1998;**125**:4325–33.
63. Alappat S, Zhang ZY, Chen YP. Msx homeobox gene family and craniofacial development. *Cell Res* 2003;**13**:429–42.
64. Cobourne MT, Sharpe PT. Sonic hedgehog signaling and the developing tooth. *Curr Top Dev Biol* 2005;**65**:255–87.
65. Sarkar L, Sharpe PT. Inhibition of Wnt signalling by exogenous Mfrzb1 protein affects molar tooth size. *J Dent Res* 2000;**79**:920–5.
66. Mandler M, Neubuser A. FGF signalling is necessary for the specification of the odontogenic mesenchyme. *Dev Biol* 2001;**15**:548–59.
67. Savill J, Fadok V. Corpse clearance defines the meaning of cell death. *Nature* 2000;**407**:784–9.
68. Deckwerth TL, Elliott CM, Knudson EM, Johnson EM, Snider WD, Korsmeyer SJ. Bax is required for neuronal death after trophic deprivation and during development. *Neuron* 1996;**17**:401–11.
69. Ohazama A, Modino SA, Miletich I, Sharpe PT. Stem-cell-based tissue engineering of murine teeth. *J Dent Res* 2004b;**83**:518–22.