Apoptosis 1997; 2: 471–477

Non-expression of insulin-like growth factor-I receptor is associated with apoptosis: an ultrastructural study on rat ameloblasts

B. K. Joseph, N. W. Savage, D. J. Harbrow and W. G. Young

Division of Oral Biology and Pathology, School of Dentistry, The University of Queensland, Brisbane, Queensland 4072, Australia

Insulin-like growth factor-I (IGF-I) is a pleiotrophic polypeptide which appears to have roles both as a circulating endocrine hormone and as a locally synthesized paracrine or autocrine tissue factor. IGF-I plays a major role in regulating the growth of cells *in vivo* and *in vitro* and initiates metabolic and mitogenic processes in a wide variety of cell types by binding to specific type I receptors in the plasma membrane. In this study, we report the distribution of IGF-I receptors in odontogenic cells at the ultrastructural level using the high resolution protein A-gold technique. In the pre-secretory stage, very little gold label was visible over the ameloblasts and odontoblasts. During the secretory stage the label was mostly seen in association with the cell membranes and endoplasmic reticulum of the ameloblasts. Lysosome-like elements in the post-secretory stage were labelled as well as multivesicular dense bodies. Very little labelling was encountered in the ameloblasts in the transitional stage, where apoptotic bodies were clearly visible. The maturation stage also exhibited labelling of the secretory-like granules in the distal surface. The presence of gold particles over the plasma membrane is an indication that IGF-I receptor is a membrane-bound receptor. Furthermore, the intracellular distribution of the label over the endoplasmic reticulum supports the local synthesis of the IGF-I receptor. The absence of labelling over the transitional ameloblasts suggests that the transitional stage may require the non-expression of IGF-I as a prerequiste or even a trigger for apoptosis.

Key words: Ameloblasts; apoptosis; electronmicroscopy; insulin-like growth factor; odontogenesis; rat incisor.

(Received 15 April 1997; accepted 26 June 1997)

This study was supported by The National Health and Medical Research Council of Australia and the Australian Dental Research Fund, Inc.

Correspondence to B. K. Joseph, Division of Oral Biology and Pathology, School of Dentistry, The University of Queensland, St. Lucia, Brisbane, Queensland 4072, Australia. Tel: (+07) 33652419; Fax: (+07) 33651109.

Introduction

In many ontogenetic sequences, insulin-like growth factors are important for cell growth and cell differentiation. Insulin-like growth factor-I is a pleiotrophic polypeptide which appears to have roles both as a circulating endocrine hormone and as a locally synthesized paracrine or autocrine tissue factor[1]. The factor plays a major role in regulating the growth of cells *in vivo* and *in vitro*[2] and initiates metabolic and mitogenic processes in a wide variety of cell types by binding to specific type I receptors in the plasma membrane. The type-I IGF receptor (IGFR-I) is a membrane-bound tyrosine kinase with significant homology to the insulin receptor[3–5] and consists of two α and two β subunits linked by disulphide bridges.[6] At present, the precise post-receptor signalling pathway of the type-I IGF receptor is not well defined. However, based on previous studies on the insulin receptor, it is assumed that the tyrosine-kinase activity of the type-I IGF receptor probably plays an important role in mediating many of the biologic actions of IGF-I.[6]

The rat incisor is unique in that, simultaneously, all stages of the complex life cycle of the ameloblasts are represented along the enamel-forming aspect of the tooth. Ameloblasts from rat incisors have been studied extensively[7–9] and a series of sequential stages of ameloblasts in an incisor can be divided into two phases. In the first phase, the cells differentiate into ameloblasts and secrete the enamel matrix. In the second phase the ameloblasts function in enamel maturation which is achieved by an increase in inorganic material and resorption of organic components and water from the enamel. The distinct interphase between the two phases corresponding to the secretion and maturation

stages is referred to as the zone of transition. Cell death at this zone has been described by Smith and Warshawsky[10] in their quantitative studies of cell turnover in the rat incisor. This zone is short and ameloblasts pass through this stage in only 19–21 h. The transition zone is characterized by programmed cell death of approximately 25% of the ameloblast population.

Growth factors have important roles in the regulation of cell division, differentiation and tissue growth. They also have an important role in the regulation of apoptosis. The viability of most cells is dependent on the presence of growth factors. Haematopoietic cells in particular are exceedingly susceptible to apoptosis if deprived of their respective trophic growth factors.[11] Similarly, withdrawal of growth factors *in vivo*, for example by hormone ablation, can induce atrophy and apoptosis. There is an increasing amount of data suggesting that growth factors acting through tyrosine kinase receptors are important survival factors suppressing apoptosis.[12] However, our understanding of the specific molecular mechanisms by which growth factors prevent apoptosis is limited.

Recent studies[13] from our laboratory have localized the distribution of IGF-I receptor in the odontogenic cells by immunohistochemistry. The distribution and intensity of IGF-IR expression varied with the phenotypic stages of the ameloblasts. The absence of staining over the transitional ameloblasts, some of which undergo apoptosis, suggests that the transitional stage may require the non-expression of IGF-I which may be a prerequiste or even a trigger for apoptosis.[13] That this status is accompanied by apoptosis of 25% of the population makes the changing distribution of the IGF-IR in the ameloblast life cycle an exciting biological observation. We have also localized the gene associated with apoptosis, sulphated glycoprotein-2 (SGP-2) in the transitional ameloblasts, where IGF-I receptor expression was found to be absent, providing further evidence for a role of IGF-I in apoptosis.[14]

Much could be learned, for example about the varying relationships of the receptor to cell organelles at the ultrastructural level, when the light level findings are of a change from a diffuse to a granular staining pattern. Is this an example of endocytotic membrane-receptor processing? What is the sequence of IGF-I receptor synthesis when the cells embark on enamel maturation after emerging from the transitional state? This curious sequence of events, with respect to the distribution of IGF-IR in the ameloblast life cycle, is clearly worthy of further investigation by immunocytochemistry at the ultrastructural level.

There is little information concerning the form of apoptosis in tooth development which occurs in highly specialized post-mitotic ameloblasts. Apoptotic bodies and associated enzyme profiles have been described in the zone and hence the main aim of this study is to investigate the distribution of the IGF-I receptor at the ultrastructural level in the odontogenic cells of the rat incisor ameloblasts. The study was planned to give further insight into the mechanism of action of IGF-I in tooth development by detecting the presence of IGF-I receptors during different stages of tooth formation.

Materials and methods

Animals

Ten, four week old male Lewis rats, obtained from the Central Animal Breeding House, The University of Queensland were used in this study. Animals were caged in a room at 22°C with a 12 h (0600–1800 h) light–dark cycle and were fed *ad libitum* with commercial rat pellets. The guidelines for animal experimentation prescribed by National Health and Medical Research Council of Australia were followed and the project had the approval of the Institutional Ethics Committee.

Electron microscopy

Animals were anaesthetized by an intra-peritoneal injection of 0.1 ml/100 g sodium pentobarbitone and perfused intracardially with phosphate buffered saline (PBS, pH 7.4), followed by 1.25% glutaraldehyde and 0.2% picric acid solution in 0.066 M cacodylate buffer, pH 7.2. The mandibles were dissected and post-fixed by immersion in the same fixative for 4 h. The tissues were then processed and embedded in L R white resin (Bio-Rad, Microscience Division, Hemel Hempstead, UK) according to the methods of Nanci *et al*.[15] Semi-thin sections (1–2 µm) in the coronal plane, transversely through the enamel organ of the incisor teeth were

cut on an Ultracut E ultramicrotome (Reichert-Jung, Austria) and stained with toluidine blue for light microscopy. Ultra-thin sections were also cut (60–70 nm) from selected blocks and stained with uranyl acetate for electron microscopy.

The high resolution protein A-gold immunocytochemical technique was followed.[15] Briefly, grid mounted tissue sections were washed with distilled water, floated on to Tris buffered saline in 1% BSA (TBS/BSA, pH 7.2) and then transferred and incubated for 2 h at room temperature on a drop of diluted rabbit anti-human IGF-I receptor antibody. The specificity of this antibody has been described elsewhere.[13,16] After incubation, sections were rinsed with TBS/BSA, followed by incubation for 60 min at room temperature with 10 nm protein A-gold complex (Bio Cell, Cardiff, UK). The tissue sections were then washed thoroughly with TBS/BSA, rinsed with distilled water and air dried. For control experiments, sections were incubated with (i) protein A-gold alone and (ii) nonimmune serum.

Results

Figure 1 shows a high magnification lightmicrograph of the transitional ameloblasts in odontogenesis. The ameloblasts decrease in height, and the zone is characterized by the presence of large, round, darkly-stained bodies in the proximal ends of the ameloblasts and in the adjacent papillary cells. Figure 2 shows an ultrastructural view of the apoptotic bodies. These cytologically identifiable bodies are the apoptotic bodies. It is noteworthy that apoptotic bodies were most discernible in this transitional zone.

Figure 1. This haematoxylin and eosin stained section shows the topography of the principal cells studied in the rat incisor. The ameloblasts (A) in the zone of transition are illustrated here. The ameloblasts (A) are reduced to approximately half their original length and apoptotic bodies (AP) (arrow heads) are found within the cytoplasm of the ameloblasts.

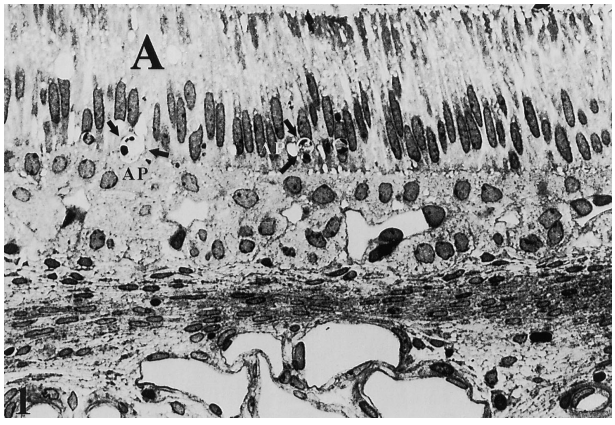
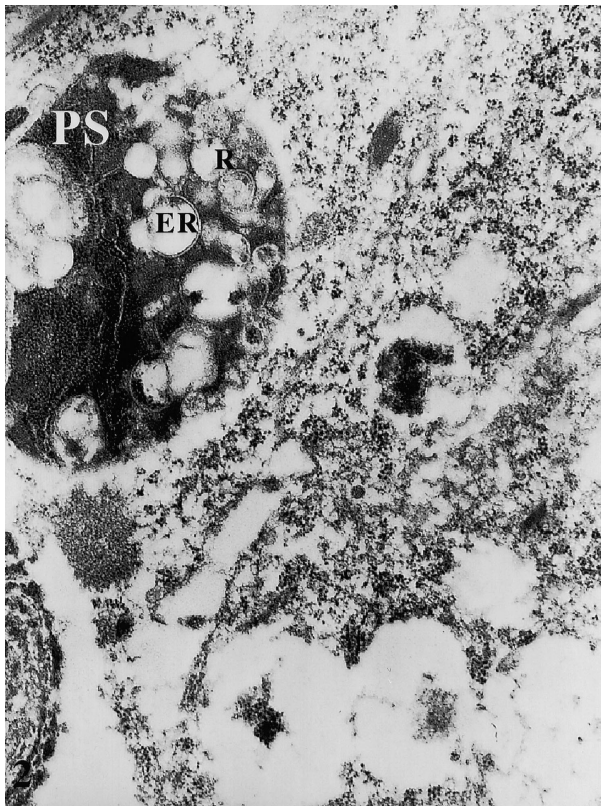
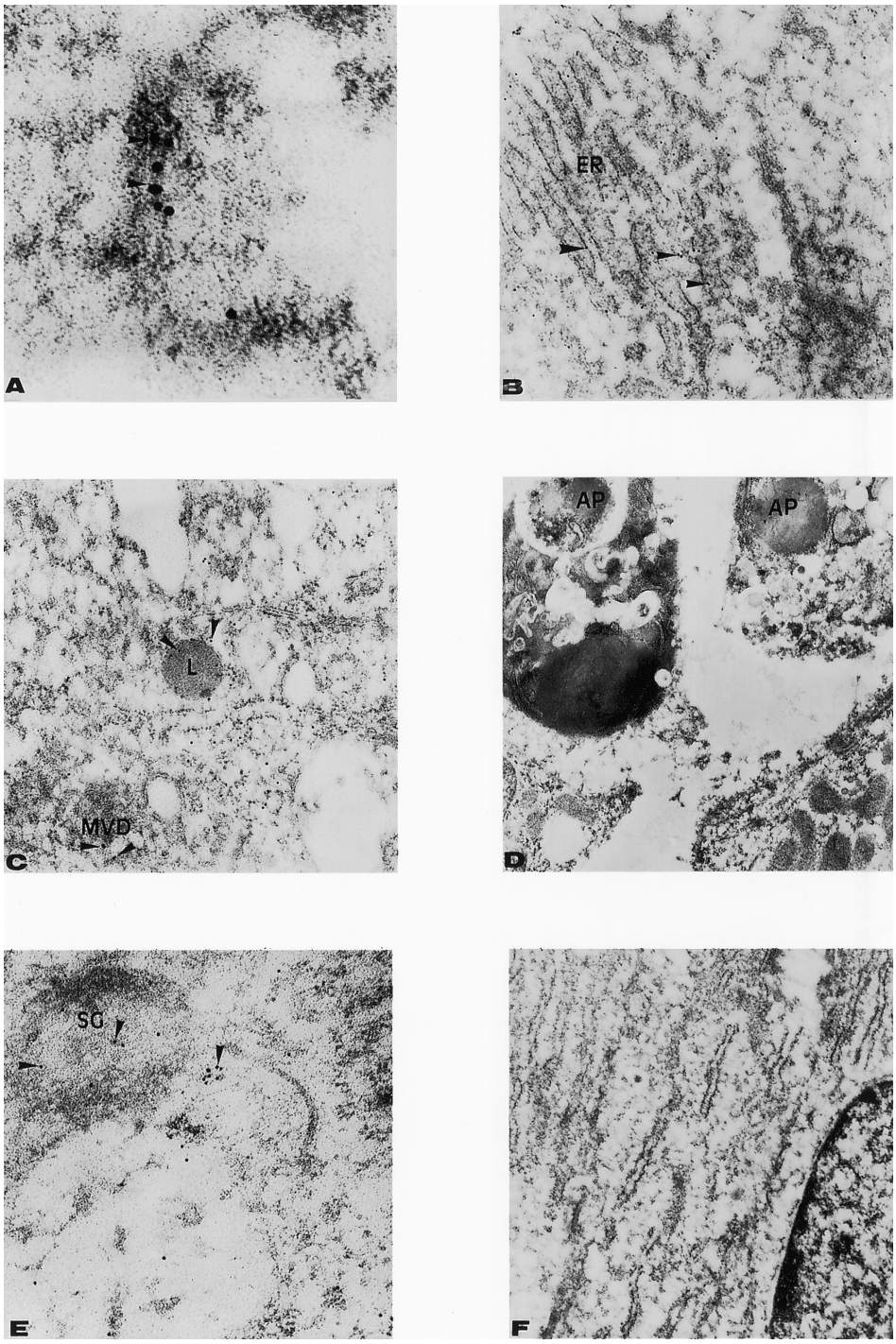


Figure 2. Ultrastructural micrograph showing phagosomes (PS) from an ameloblast in the transitional zone. These are heterophagosomes and contain ribosomes (R) and profiles of endoplasmic reticulum (ER).



At the ultrastructural level, gold particles were found in ameloblasts involved in enamel secretion and maturation and was definitively localized in specific cytoplasmic organelles. Only low background labelling was present over the nuclei and extracellular matrix. In the pre-secretory stage, very little gold label was visible over the pre-ameloblasts and pre-odontoblasts (data not shown). During the secretory stage, the label was mostly seen in association with the outer cell membranes (Figure 3A) and endoplasmic reticulum (Figure 3B) of the ameloblasts. Lysosome-like elements of the post-secretory stage were labelled as were multivesicular dense bodies (Figure 3C). Only background level

Figure 3. Ultrastructural localization of IGF-I receptor in rat ameloblasts using gold label. (**A**) Electron micrograph of an ultra-thin section of the rat incisor tooth in the secretory stage. The section was labelled with rabbit antiserun to IGF-I receptor and thereafter with protein A-gold complex (10 nm). Gold label is present (arrowheads) over the outer cell membranes of the ameloblasts. (**B**) Electron micrograph of an ultra-thin section in the secretory stage of tooth development. Gold label is present over the endoplasmic reticulum (ER) (arrowheads). (**C**) Electron micrograph of an ultra-thin section in the post-secretory stage of odontogenesis. Lysosome-like elements (L) and multivesicular dense bodies (MVD) showing gold labelling (arrowheads). (**D**) Electron micrograph of an ultra-thin section from the rat incisor in the zone of transition. Transitional stage ameloblasts showing apoptotic bodies (AP) engulfed in a phagosome. There was no gold label visible at this stage. (**E**) Electron micrograph of an ultra-thin section from the rat incisor. IGF-I receptor is visualized by immuno-gold staining technique. Gold label is present in the secretory granules (SG) in the maturation stage of tooth development. (**F**) Electron micrograph of an ultra-thin section of the rat incisor. The section has been immuno-gold stained by omitting incubation with antiserum to IGF-I receptor. There was no gold label visible.



labelling was found in the ameloblasts in the transitional stage, and apoptotic bodies were not specifically labelled (Figure 3D). In contrast, the ameloblasts of the maturation stage exhibited labelling in granular bodies associated with the surface of the maturing enamel (Figure 3E). Control sections in this study showed no labelling (Figure 3F).

Discussion

Insulin-like growth factor receptor has been demonstrated in a wide variety of tissues and cell lines by competitive binding and affinity crosslinking studies[17–21] and more recently by means of molecular hybridization techniques. This suggests that IGF-I has widespread and varying effects. Resnicoff *et al.*[22] have demonstrated a definite role for IGF-I receptor in programmed cell death in various tumour cell lines. They found that a decrease in the number of IGF-IRs causes massive apoptosis *in vivo* in several transplantable tumours, either from humans or rodents.

The concentration of gold label within the organelles of secretory and maturation ameloblasts has enabled us to explain the pattern of staining found with the IGF-I receptor antibody at the light microscopic level. The presence of gold particles over the plasma membrane in secretory ameloblasts is an indication that IGF-I receptor is a membrane-bound receptor. Furthermore, the intracellular distribution of the label over the endoplasmic reticulum suggests that there is local synthesis of the IGF-I receptor. The presence of label in lysosomes and multivesicular bodies in the post-secretory stage suggests that the granular deposits seen in the post-secretory ameloblasts at the light level[13] represent internalization of the receptor.

By quantitative electron microscopic radioautography and subcellular fractionation, a number of investigators have obtained clear evidence for IGF-IR internalization[23,24] and recycling.[24,25] Thus the IGF-I-receptor complex is not only mobile at the plasma membrane but also moves through the cell volume by endocytosis. In a part of this journey, the IGF-I-receptor complex remains intact, but at a certain point the complex enters an acidic environment that results in its dissolution. The receptor is then recycled back to the cell surface whereas the ligand is degraded.[26–28] Like that of other peptide hormones, gold labelling for IGF-I receptor in the cytoplasm during odontogenesis may relate to receptor-mediated internalization and recycling, or it might represent an intracellular site for the action of IGF-I.

Whereas it is widely accepted that binding of the IGF-I ligand to the receptor extracellular domain results in activation of its tyrosine kinase domain, the molecular mechanism by which this event is then transduced into specific biologic responses still remains elusive.[4] Receptor aggregration has been proposed as a possible mechanism by which the insulin receptor[29] and the epidermal growth factor receptor[30] initiate the cascade of intracellular events, which will eventually culminate in a biological effect. Similarly, recent studies suggest that receptor-mediated endocytosis of the IGF-I ligand and receptor aggregate represent a potential mechanism by which IGF-I action is exerted.[28] Thus, internalization of the receptor-ligand complex may be occurring continually during enamel matrix secretion.

Accordingly, the change in pattern of the gold labelling of ameloblasts which are now terminating enamel matrix secretion, and the gradual reduction of gold label prior to the non-expression of IGF-I receptor in the transitional zone, is a finding of intrinsic significance. It may be that this relates to the slowing down of matrix secretion or that the IGF-I receptor may be sequestered and broken down by lysosomes as the events of transition approach. Conceivably this phenomenon could result in the observed elimination of IGF-I receptor expression in the transitional zone. An alternative explanation for this could be that an inhibition of receptor biosynthesis occurs in cells entering the transitional stage.

Non-expression of the IGF-I receptor in transitional ameloblasts is also accompanied by apoptosis. Approximately 25% of the ameloblast population undergoes programmed cell death in the normal life cycle of the ameloblast.[39,40] This mostly occurs in the region of post-secretory transition which occupies a period of 19 h in the 30-day life-cycle of the ameloblast population.[10] At the light microscopic level, apoptotic ameloblasts can be recognized readily as pyknotic nuclear fragments within and between adjacent ameloblasts or in the stratum intermedium.[39,41] Ultrastructurally, the ef-

fete ameloblasts are phagocytosed by their neighbours and appear as phagolysosomes within the ameloblast cytoplasm.[41,42] The activities of lysosomal enzymes such as dipeptidyl peptidase II in this zone have been described.[42] Various studies have shown that the enzyme, tissue transglutaminase, is a reliable marker for cells undergoing apoptosis.[43,44,45]

The absence of labelling over the transitional ameloblasts suggests that the transitional stage may require the non-expression of IGF-I as a prerequiste or even a trigger for apoptosis. Accordingly, it is tempting to speculate that apoptosis of a proportion of the transitional ameloblasts is one further example in developmental biology of programmed cell death associated with a depletion of endocrine stimulation. In this case it would be depletion of IGF-I, predicated upon by down-regulation of its receptor.

It is unknown how apoptosis of ameloblasts is triggered. However, we have shown by *in situ* hybridization, that the mRNA for sulphated glycoprotein-2 (SGP-2), a marker protein for apoptosis is expressed specifically over the transitional zone.[14] The expression of several oncogenes and tumour suppressor genes such as c-myc, bcl-2, fas and p53 are known to modulate apoptosis in various tissues.[46] The interaction of intracellular proteins modulate the apoptotic process. Several of these proteins have been identified including p35, bcl-2 and its related family members (*e.g.*, bclx, bax and bak) and the bcl-2 binding protein, bag-1. Involvement of these oncogenes in the apoptotic processes of ameloblasts remains to be clarified.

Apoptosis[31] is a basic cellular phenomenon whereby cells are deleted from living tissues in a physiological manner.[32] Apoptosis is a feature of several and, perhaps, all cell and tissue systems and occurs when it appears necessary or beneficial for the host organism.[33] Moreover, some growth factors and hormones have been shown to induce or suppress apoptosis, as well as to stimulate mitosis.[34–37] There is also evidence that apoptosis is an active process of gene-directed cellular self-destruction and that in most of the circumstances in which it occurs, it serves a biologically meaningful, homeostatic function.[33,38] Thus, apoptosis of ameloblasts deserves further investigation as to its relation to its possible growth factor dependence.

References

1. Daughaday WH, Rotwein P. Insulin like growth factor I and II. Peptide, messenger ribonucleic acid and gene structures, serum, and tissue concentrations. *Endocr Rev* 1989; 10: 68–91.
2. Froesch ER, Schmid C, Schwander J, Zapf JA. Actions of insulin-like growth factors. *Annu Rev Physiol* 1985; 47: 443–467.
3. Steele-Perkins G, Turner J, Edman JC, *et al*. Expression and characterization of a functional human insulin-like growth factor I receptor. *J Biol Chem* 1988; 263: 11486–11492.
4. Czech MP. Signal transmission by the insulin-like growth factors. *Cell* 1989; 59: 235–238.
5. Rechler MM, Nissley SP. Insulin-like growth factors. In: Sporn MB, Roberts AB, eds. *Peptide Growth Factors and Their Receptors I*. New York, NY (USA): Springer-Verlag, 1990: 263–368.
6. LeRoith D, Roberts CT Jr. Insulin-like growth factor I (IGF-I): A molecular basis for endocrine versus local action? *Mol Cell Endocrinol* 1991; 77: C57–C61.
7. Warshawsky H, Smith CE. Morphological classification of rat incisor ameloblasts. *Anat Rec* 1974; 179: 423–446.
8. Sasaki T. Cell biology of tooth enamel formation. Functional electron microscopic monographs. Karger, Basel: *Monographs in Oral Science*, 1990: Vol. 14.
9. Joseph BK, Savage NW, Young WG, Gupta GS, Breier BH, Waters MJ. Expression and regulation of insulin-like growth factor-I in the rat incisor. *Growth Factors* 1993; 8: 267– 275.
10. Smith CE, Warshawsky H. Quantitative analysis of cell turnover in the enamel organ of the rat incisor. Evidence for ameloblast death immediately after enamel matrix secretion. *Anat Rec* 1977; 187: 63–98.
11. Rubin R, Basera R. Insulin-like growth factor-I receptor: its role in cell proliferation, apoptosis and tumourgenicity. *Lab Invest* 1995; 73: 311–324.
12. Collins MK, Perkins GR, Rodriguez-Tarduchy-G, Nieto-MA, Lopez-Rivas-A. Growth factors as survival factors: regulation of apoptosis. *Bioessays* 1994; 16(2):133–138.
13. Joseph BK, Savage NW, Young WG, Waters MJ. Insulin-like growth factor-I receptor in the cell biology of the ameloblast: an immunohistochemical study. *Epi Cell Biol* 1994a; 3(2): 47–53.
14. Joseph BK, Gobé GC, Savage NW, Young WG. Expression and localization of sulphated glycoprotein-2 mRNA in the rat incisor tooth ameloblasts: relationships with apoptosis. *Int J Exp Path* 1994b; 75: 313–320.
15. Nanci A, Mckee MD, Smith CE. Immunolocalization of enamel proteins during amelogenesis in the cat. *Anat Rec* 1992; 233: 335–349.
16. Rosenzweig SA, Zetterstrom C, Benjamin A. Identification of retinal insulin receptors using site-specific antibodies to a carboxyl-terminal peptide of the human

insulin receptor α-subunit. *J Biol Chem* 1990; 265: 18030–18034.
17. Lee PDK, Rosenfield RG, Hintz RL, Smith SD. Characterization of insulin, insulin like growth factors I and II and growth hormone receptors on human leukemic lymphoblasts. *J Clin Endocrinol Metab* 1986; 62: 28–34.
18. Pepe MG, Ginzton NH, Lee PDK, Hintz RL, Greenberg PL. Receptor binding and mitogenic effects of insulin and insulin-like growth factors I and II for human myeloid leukemic cells. *J Cell Physiol* 1987; 133: 219–223.
19. Trippel SB, Chernausek SD, Van Wyk JJ, Moses AC, Mankin HJ. Demonstration of type I and type II somatomedin receptors on bovine growth plate chondrocytes. *J Orthpaed Res* 1988; 6: 817–822.
20. Aron DC, Rosenzweig JL, Abboud HE. Synthesis and binding of insulin-like growth factor I by human glomerular mesangial cells. *J Clin Endocrinol Metab* 1989; 68: 585–589.
21. Centrella M, McCarthy TL, Canalis E. Receptors for insulin-like growth factors I and II in osteoblast-enriched cultures from fetal rat bone. *Endocrinol* 1990; 126: 39–44.
22. Resnicoff M, Abraham D, Yutanawiboonchai W, *et al*. The insulin-like growth factor I receptor protects tumor cells from apoptosis in vivo. *Cancer Res* 1995; 55: 2463–2469.
23. Posner BI. *Polypeptide Hormone Receptors*. New York, NY (USA): Dekker, 1985.
24. Carpentier JL. The journey of the insulin receptor into the cell: from cellular biology to pathophysiology. *Histochem* 1993; 100: 169–184.
25. Posner BI, Bergeron JJ, Josefsberg Z, *et al*. Polypeptide hormones intracellular receptor and internalization. *Rec Prog Horm Res* 1981; 37: 539–582.
26. Schalch DS, Sessions CM, Farley AC, Masakawa A, Emler CA, Dills DG. Interaction of insulin-like growth factor I/somatomedin-C with cultured rat chondrocytes: receptor binding and internalization. *Endocrinol* 1986; 118: 1590.
27. Bhaumick B, Bala RM. Receptors for insulin-like growth factors I and II in the developing mouse limb bud. *Biochem Biophys Acta* 1987; 927: 117–128.
28. Werner H, Woloschak M, Stannard B, Shen-Orr Z, Roberts CT Jr, LeRoith D. The insulin-like growth factor I receptor: Molecular biology, heterogeneity and regulation. In: LeRoith D, ed. *Insulin-like Growth Factors: Molecular and Cellular Aspects*. Boston, MA (USA): CRC Press, 1991: 17–47.
29. Kahn CR, Baird KL, Jarrett DB, Flier JS. Direct demonstration that receptor crosslinking or aggregation is important in insulin action. *Proc Natl Acad Sci USA* 1978; 75: 4209– 4212.
30. Schlessinger J. Signal transduction by allosteric receptor oligomerization. *Trends Biochem Sci* 1988; 13: 443–446.
31. Kerr JFR, Wyllie AH, Currie AR. Apoptosis: a basic phenomenon with wide ranging implications in tissue kinetics. *Br J Cancer* 1972; 26: 293–297.
32. Wyllie AH, Kerr JF, Currie AR. Cell death: The significance of apoptosis. *Int Rev Cytol* 1980; 68: 251–253.
33. Kerr JFR, Harmon BV. Apoptosis: An historical perspective. In: Tomei LD, Cope FO, eds. *Apoptosis: The Molecular Basis of Cell Death. Current Communications in Cell & Molecular Biology, 3*. Cold Spring Harbor, NY (USA): Cold Spring Harbor Laboratory, 1991: 5–17.
34. Sandow BA, West NB, Norman RL, Brenner RM. Hormonal control of apoptosis in hamster uterine luminal epithelium. *Am J Anat* 1979; 156: 15–36.
35. Wyllie AH, Morris RG. Hormone-induced cell death. Purification and properties of thymocytes undergoing apoptosis after glucocorticoid treatment. *Am J Pathol* 1982; 109: 78–87.
36. Duke RC, Cohen JJ. IL-2 addiction: Withdrawal of growth factor activates a suicide program in dependent T cells. *Lymphokine Res* 1986; 5: 289–299.
37. Williams GT, Smith CA, Spooner E, Dexter TM, Taylor DR. Haemopoietic colony stimulating factors promote cell survival by suppressing apoptosis. *Nature* 1990; 343: 76– 78.
38. Cohen JJ. Overview: Mechanisms of apoptosis. *Immunol Today* 1993; 14: 126–130.
39. Smith CE. Ameloblasts: Secretory and resorptive functions. *J Dent Res* 1979; 58B: 695– 706.
40. Warshawsky H. Ultrastructural studies on amelogenesis. In: Butler WT, ed. *The Chemistry and Biology of Mineralized Tissues*. Birmingham, AL (USA): Ebsco Media, 1985: 33–45.
41. Moe H, Jessen H. Phagocytosis and elimination of amelocyte debris by stratum intermedium cells in the transitional zone of the enamel organ of the rat incisor. *Z Zellforsch Mikrosk Anat* 1972; 131: 63–75.
42. Smid JR, Monsour PA, Rousseau EM, Young WG. Cytochemical localization of dipeptidylpeptidase II activity in rat incisor tooth ameloblasts. *Anat Record* 1992; 233: 493–503.
43. Piacentini M, Autuori F, Dini L, *et al*. 'Tissue' transglutaminase is specifically expressed in neonatal rat liver cells undergoing apoptosis upon epidermal growth factor-stimulation. *Cell Tissue Res* 1991; 263: 227–235.
44. Alaoui S El, Mian S, Lawry J, Quash G, Griffin M. Cell cycle kinetics, tissue transglutaminase and programmed cell death (apoptosis). *FEBS Lett* 1992; 311: 174–178.
45. Knight RL, Hand D, Piacentini M, Griffin M. Characterization of the transglutaminase-mediated large molecular weight polymer from rat liver; its relationship to apoptosis. *Eur J Cell Biol* 1992; 60: 210–216.
46. Sugerman PB, Joseph BK, Savage NW. The role of oncogenes, tumour suppressor genes and growth factors in oral squamous cell carcinoma: a case of apoptosis versus proliferation. *Oral Diseases* 1995; 1: 172–188.