Expression of Desmosomal Proteins and Their Implications During Enamel Organ Morphogenesis

Hadi A. Bilal
Department of Oral Pathology, Faculty of Dental, Al Andalus University for Medical Sciences, Tartus, Syria

Abstract

Background/Objective: The present article is between the fewest that describe the expression of desmosomal proteins in human tooth organ and to implicate desmosome and its components in tooth morphogenesis. Although present in all epithelia, desmosomes are particularly down regulated in undifferentiated, dedifferentiated and malignant cells. Methodology: In the present article, using immunohistochemical staining, we report the expression patterns of 6 desmosomal components; Desmoplakin, plakoglobin, desmoglein, plakophilin-1, plakophilin-2, and plakophilin-3. The dental, oral and ectodermal epithelia were investigated in 7 and 9 weeks’ human embryos. Results: The expression of these components appeared to associate with cell differentiation, stage of development and epithelial type. The fundamental desmosomal proteins, desmoplakin, plakoglobin and desmoglein, produced almost identical profiles, suggesting that desmosomes are absent or under developed in negative and weak reacting areas respectively. Whilst the accessory component plakophilin-1 was always absent, plakophilin-2 and -3 were strongly labeled in the embryonic epidermis and moderately expressed in the oral epithelium. In the developing enamel organ, gradients of fundamental protein expressions were produced being strong towards the oral epithelium and weak/negative around the inner enamel epithelium. These gradients were also produced using antibodies to plakophilin-2 and -3 although the majority of enamel organ cells were unlabeled. Conclusion: Mirror image symmetry was found both for the maxillary and mandibular tooth germs, thus bringing further evidence implicating desmosome and its components in the mechanisms regulating tooth morphogenesis.

Keywords: Asymmetry, desmosomes, enamel organ, expression, morphogenesis

Introduction

Mammalian teeth are initiated from the oral ectoderm which is a primitive stratified squamous epithelium. The early stages of tooth development are regulated by interactions between this epithelium and the underlying mesenchymal tissue, through antagonistic signaling involving the growth factors, fibroblast growth factors (FGFs), and bone morphogenetic proteins (BMPs). Epithelial FGF-8 stimulates BMP-2, and BMP-4 inhibits Pax-9 expression in the mouth branchial arch mesenchyme. This antagonistic signaling was suggested to determine the sites of tooth buds. Pax-9, however, is the only gene performing this function because tooth buds develop in normal locations in Pax-9 knockouts. Indeed, other genes are more restricted to the dental lamina, such as the transcription factor Pitx-2, the gene behind Rieger syndrome comprising missing teeth, Shh, both of which subsequently become restricted to the forming tooth buds.[1,2] The mesenchymal component of teeth derives from neural crest in the midbrain region. In the case of mammalian dentition, four types of teeth are usually considered to form: Incisor, canine, premolar, and molar teeth within each vestibular lamina (responsible for the formation of permanent teeth, while no premolars are seen in the lacteal teeth). Tissue recombination experiments give an indication that early dental epithelium, and not neural crest mesenchyme, determines tooth type.[3] The signaling molecules are the same as in the determination of tooth regions. As neural crest cells in all locations are equally responsive to BMP-4 and FGF-8, it was concluded that the cells are determined by dynamic patterns.
of signal molecules expressed by the early facial processes.\textsuperscript{[4]}
Conceivably, the differential activation of key transcription factors results in specific combinations of homeobox genes which determine tooth identity. FGFs induce the expression of the homeobox genes Lhx-6 and -7, Msx-1, and Dlx-1 and 2; BMPs upregulate Msx-1 and 2 and Dlx-2; and Shh indices Gli-1, 2, and 3.\textsuperscript{[2-5]}

Although it has been shown that said genes are essential for tooth morphogenesis, it is reasonable to suggest that these factors act through the modulation of fundamental cellular processes including cell growth, differentiation, and adhesion. Among the major intercellular junctions, desmosomes are believed to stabilize and strengthen cell–cell adhesion in epithelia subjected to mechanical stress including the epidermis and the oral epithelium. They are also involved in the spatial organization of intermediate-sized filaments of the cytoskeleton (intermediate filaments [IFs]).\textsuperscript{[6-8]} Depending on the epithelial type, desmosomes vary in size, with diameters ranging from approximately 0.1–2 µm. However, they shared a basic ultrastructural organization, in which the plasma membranes of two adhering cells are separated from each other by a 20–30-mm wide filamentous material called desmoglea and underlaid with a dense 10–40 mm thick subplasmalemmal cytoplasmic plaque toward which bundles of IFs converge.\textsuperscript{[9]} The desmoglea contains the extracellular domain of transmembrane glycoproteins of the cadherin superfamily of cell–cell adhesion molecules.\textsuperscript{[10,11]} Desmosomal cadherins, desmocollins, and desmogleins (Dgs) mediate the adhesive function of the desmosome.\textsuperscript{[12,13]} The plaque domain contains nonglycosylated proteins including desmoplakins-1 and 2 (Dps) which anchors IFs\textsuperscript{[14,15]} and plakoglobin (Pg) which binds cytoplasmic domains of desmosomal cadherins.\textsuperscript{[16-19]} So far, at least 15 molecules have been localized to this junction. However, only Dp-1 is considered fundamental, a component present in all tissues forming desmosomes and whose absence prevents desmosome formation. This makes it the desmosomal marker par excellence. Several other proteins appear to be accessory, and their expression may be related to cell differentiation, type, or function. Among these molecules, the plakophilins (PKPs-1, 2, and 3) are members of the armadillo family implicated in adhesion and signaling.

Desmosomes are dynamic structures whose regulation involves serine and threonine phosphorylation leading to changes in epithelial structural stability.\textsuperscript{[20-22]} During epithelial regeneration\textsuperscript{[23]} and carcinogenesis of different tissues\textsuperscript{[24-26]} including the oral mucosa,\textsuperscript{[27]} partial or complete loss of these junctions was observed. This change in cohesion is probably a prerequisite for cell rearrangement, invasion, and metastasis formation.

In the enamel–dental organ, the stellate reticulum is so termed because cells are stretched into a star shape due to the presence of desmosomes that interconnect between these cells. It is suggested that the downregulation of the studied adhesion molecules is essential to the disconnection of the stellate reticulum cells.\textsuperscript{[28]} Furthermore, it has been demonstrated that a compound heterozygous desmoplakin mutations caused horizontal lines of enamel dysplasia in female patient.\textsuperscript{[29]} After all, desmosomes appear to be essential for proper enamel development. Although desmosome downregulation was described during the development of certain epithelial organs, only Pg (a protein expressed by all adhesion junctions) and Dg were investigated in mouse dental tissues.\textsuperscript{[30]} The present study is the first to report the expression patterns of Dp, Pg, Dg, PKP-1, and PKP-3 in human oral and dental epithelia, using monoclonal antibodies to human antigens and to implicate desmosomes in enamel organ morphogenesis.

**Methodology**

**Studied tissues and number of biopsy**
A total of 12 human embryos at different ages, 7–9 weeks, were used in the present study of desmosomal protein expression. The materials were obtained from the Department of Foeto-Placentology, Al-Tawleed Hospital, Damascus University, with signed consent of the mother after legally approved medical abortion. The age of the embryo was determined by the medical assessment of the date of the last period and embryo morphologic characteristics.

**Applied technique**
Precedent studies used various immunohistochemical techniques on fixed specimens. Although the tissue morphology was well preserved, the immunoreactivity was seriously compromised for all antibodies, particularly in tissues weekly expressing the studied antigen (see results). Attempts to retrieve activity using variable techniques were only partially successful.

To avoid antigen masking and modifications due to fixation and labeling procedures, we used the indirect immunofluorescence, a sensitive and reproducible technique on unfixed tissues. The tissues were snap frozen and stocked in liquid nitrogen until use. Histological sections (6 µm) were cut, air-dried for 3 h, and stored at −20°C. The histology (presence or not of tooth germ) was checked every ten sections using the toluidine blue stain [Annex; Histo. 1 and Histo. 2]. The specimens were labeled under standard conditions. Briefly, sections were incubated for 60 min with monoclonal antibodies from Progen, Biotechnik, Heidelberg (Anti-Pg, PG-5.1; anti-Dg, DG-3.1; anti-desmoplakin 1 and 2, Dp I and II-2.15; anti-plakoglobin-1, PP1-5C2; anti-plakoglobin-2 isoforms a and b, PP2/62/86/150; and anti-plakoglobin-3, 310.9.1)]. The antibodies were used at the manufacturer recommended dilutions in PBS. After thorough washing, the sections were incubated with goat anti-mouse immunoglobulins labeled with FITC (Southern Technology, Birmingham, AL, USA) for 60 min at 37°C. After washing, slides were mounted using an aqueous mountant (Fluoprep, Biomérieux, Marcy l’Etoile, France). In a negative control, normal, nonimmune, goat serum was used in place of the primary antibodies, and human epidermis was used as a positive control.
**Visualization**

Cell labeling was examined using a Leitz DMRB microscope equipped with epifluorescence illumination. Photomicrographs were taken using oil-immersion PL Fluotar objectives and automatic photoequipment controlling exposure time.

**Results**

Desmosomal protein expression varied considerably in different tissues and within individual epithelial structures, and PKP-1 was always negative in the epithelia. The distribution patterns were particularly interesting in the epithelial oral and dental tissues as well as in the epidermis.

In the present study, we report protein distribution in the dental epithelium as well as the stratified oral epithelium.

**Distribution in stratified squamous epithelium**

The oral epithelium is made of a polarized complex epithelium representing a gradient of differentiation from the basal cell layer to the superficial cell layer. This gradient is particularly interesting *in situ* studies of cell differentiation. Figure 1 presents the topographical distribution of the desmosomal components in the skin [Figure 1a-e] and in the oral mucosa [Figure 1f-j].

**Desmoplakin**

Desmoplakin was mainly localized in the suprabasal cell layers of the epidermis [Figure 1a], the oral epithelium [Figure 1f] and the tongue epithelia. The basal cells were weakly stained or negative. In the dorsal mucosa of the tongue, only the most superficial layers were strongly stained. The reaction of the monoclonal Dp I and II was apparently reduced when using fixed tissues.

**Plakoglobin and desmoglein**

The expression of Pg [Figure 1b and g] and Dg [Figure 1c and h] was almost identical to Dp stain, although the lingual labeling was remarkably strong for the Pg antibody.

**Plakophilins**

Plakophilin 1 expression was always negative in the different studied epithelium (Data not shown). The PKP-2 and -3 were very weak or negative in the oral epithelium. PKP-2 was mainly labeled in the basal cells of the epidermis [Figure 1d] and the lingual epithelium [Figure 1i], whereas in the oral epithelium, the labeling was very weak. Although present in basal and differentiating cells, PKP-3 was best detected in suprabasal cells of the epidermis [Figure 1e]. The lingual epithelium was generally very weakly stained. The oral epithelium, in contrast, showed strong reactions limited to vestibulomandibular areas [Figure 1j].

**Distribution in dental epithelia (tooth organ)**

The variation in protein profiles was correlated to site and stage of tooth development. In the following section, the distribution patterns are reported in tooth germ at different stages of morphogenesis: Dental lamina, bud stage, and cap stage (these three stages are classified according to the shape of the enamel organ). In the first two stages, the epithelial cells can morphologically be typed as either basal or suprabasal cells, where basal cells are lining the outer border of the enamel organ and separated from the mesenchymal part of tooth germ by a basal membrane. The suprabasal cells are located in the center of the dental lamina and the tooth organ. These cells start differentiating into stellate reticulum cells around organizing primary enamel knot.
Expression during dental lamina stage

The dental lamina is made of basal cells and suprabasal cells that give the epithelial thickness in this stage. Although a strong suprabasal labeling was found for Dp [Figure 2a and c], not all suprabasal cells were equally stained for Dg and Pg [Figure 2b and d]. Basal cells were weakly positive for Dp and negative for Dg and Pg proteins. PKP antibodies reaction was generally weak in the suprabasal cells to negative in the basal cells [Figure 2e and f].

Expression during bud stage

Intensely labeled cells form an eccentric longitudinal lamina being rather medially located in the budding tooth germ. The basal cells were generally weakly stained for the three proteins. Surprisingly, the basal cells of the lateral (distal) side were stained for Dp at the basal plasma membrane, but no similar reaction was found in the medial (Mesial) basal cells [Figure 2a and c]. The expression of Pg repeats that of Dp but in a weaker manner [Figure 2c and d]. PKP antibodies produced very weak reaction in these epithelial cells and PKP-1 was negative. In spite of the weak PKP-2 and 3 expressions in the oral epithelium and enamel organ, it was possible to anticipate a gradient of expression similar to that reported for the major desmosomal proteins [Figure 2e and f].

In this stage, a lateral (distal)–medial (mesial) asymmetry was observed. Thus, the proteins were more abundant in the medial rather than in the lateral side.

Expression during cap stage

Far before cytological differentiation, the changing morphology of enamel organ into cap shape is implicated in definitive tooth type. During this stage, the asymmetric distribution of the studied desmosomal proteins was more evident. The growing enamel organ exhibited larger unstained areas, particularly in the lateral and inner aspects of the cap. This was particularly clear for the Dp [Figure 2c], Pg [Figure 2d]
and Dg (not shown). The major part of the enamel organ was unstained for PKP-2 [Figure 2e] and PKP-3 [Figure 2f] although profiles related to those described in the bud stage could be detected. Moreover, as in the bud stage, the inner and outer enamel epithelial cells were the least labeled cells for all three studied proteins.

**Discussion**

It is generally accepted that cell–cell adhesion is fundamental in morphogenesis. However, only limited information dealing with desmosomal adhesions in developing oral and dental tissues are available. Among the well-characterized cell–cell adhesion components in man and mice, P-cadherin and E-cadherin expression was shown to be highly regulated both spatially and temporally during tooth development and amelogenesis.[31-33] In the early stage of molar tooth bud development, basal epithelial cells expressed both E-cadherin and P-cadherin, whereas suprabasal cells expressed only E-cadherin. At the cap stage, in addition to the cells of the inner and outer enamel epithelial cells which outline the enamel organ, the enamel knot (thought to control tooth morphogenesis) strongly expressed P-cadherin.[31,34] The expression of P-cadherin was prominent in the inner enamel epithelial cells during the early to mid-bell stage and was also evident in the nondividing cell masses at future cusp tips which are the so-called secondary enamel knots. In the tooth germ, at the late bell stage, when the cells of the inner enamel epithelial cells began to polarize and to differentiate into ameloblasts, the polarizing ameloblasts lost P-cadherin and strongly expressed E-cadherin. However, E-cadherin was also lost from polarized ameloblasts at later stages. The stratum intermediate and the stellate reticulum were E-cadherin positive from the bell stage onward even at the stages when the ameloblasts became E-cadherin negative again. These results suggest that the differential expression of cadherins plays a role in the regulation of tooth morphogenesis. In contrast, alteration of E-cadherin expression during later stages of tooth development is related to differentiation and function of the ameloblasts and other cells supporting amelogenesis.[31-33] Furthermore, it is known that ameloblasts progression needs several developmental stages that involve contact, detachment, movement, and reattachment to each other.[35] It is interesting to note that during all stages of tooth morphogenesis reported in our study, the desmosomal markers were negative to weakly expressed where P-cadherin is positive including the inner enamel epithelial cells, enamel knot, and basal cells. This makes us suggest a mutually exclusive relation between P-cadherin and desmosome-based cell–cell adhesion. This is further supported by the fact that P-cadherin is strictly found in the basal cells of adult epidermis where desmosomes are least abundant and most immature. In contrast, the relationship between E-cadherin and desmosome formation appear to be more complex, since positive cells forming adherence junctions may or may not express the desmosomal markers. This does not contradict the findings of Lewis et al. that E-cadherin-based adhesion may accelerate desmosome assembly in cell culture.[36]

Among all desmosomal components (currently more than 15), the distribution of Dg and Pg in the developing and growing enamel organ was reported in a study by Fausser et al. in mice.[39] As reported in that study, the authors described an asymmetric distribution of these components between the lateral (distal) and medial (mesial) outer enamel organ. Compared to our results [Figure 3], the adjacent suprabasal cells did not exhibit such asymmetry, and interestingly, the distribution pattern in the outer enamel epithelial cells was reversed, that is, strong in the lateral (distal) and weak in the medial (mesial) side. It is tempting to suggest that this medial-lateral reversal could reflect species-specific variations. However, the role of desmosome in stabilizing a given morphological pattern during development is well known. It has shown that any change in normal expression in some adhesion molecules like nectin-1 or nectin-3 result in small and less numerous desmosomes between ameloblasts and the stratum intermedium, which could alter the enamel organ development.[37,39] Other types of cell–cell junctions based on E-cadherins and p-cadherins may be required to maintain the contact between epithelial cells at particular sites actively engaged in morphogenic events such as rearrangement, proliferation, and migration. A recent study suggested that the tooth-specific MMP (Mmp20) facilitates the movement of ameloblasts during dental enamel development.[35] Interestingly, Mmp20 (enamelysin) null mice have thin, brittle enamel with a dysplastic rod pattern.[40] These findings emphasize the importance of desmosomal regulation not only during morphogenesis but also during enamel organ differentiation and amelogenesis that could probably alter the related odontogenesis. We, thus, propose to investigate the correlation between adhesion molecules and the Mmp20 expression during tooth development.

It is well established in cell culture that desmosomes formation passes by different stages of maturation and stability.[41] Similarly, in situ, desmosomes are of small size, immature, and few in simple epidermis, in developing organs and in actively growing cells at wound edges.[42] In stratified squamous epithelia, basal cells are progenitors which resemble simple epithelia and developing organs by having immature unstable desmosomes. In contrast, the differentiating and postmitotic suprabasal keratinocytes form large and mature desmosomal contacts.[43] In the enamel organ, we showed that expression patterns of individual molecules making up the desmosomal structure vary considerably. Thus, PKPs which are considered accessory desmosomal proteins,[44] were the least expressed, and PKP-1 being absent. PKP-1 is thought to be implicated in the terminal stabilization of desmosomes in the epidermis by the ability to link keratin IFs to the cytoplasmic plaque domain of desmosomes.[45] The expression of PKP-2 in the epidermis was restricted to basal cells.[46] In the enamel organ, the basal and suprabasal cells were positive, and in all cells, the reaction was
weak. This suggested that the cells of the two compartments are in a dedifferentiation state probably necessary for growth and phenotype changes.

Taken together with the observed gradients in protein expression, we suggest the existence of hierarchy in cell–cell adhesion stability. According to the labeling patterns, we propose the Pg- and Dp-negative areas as highly active zone lacking desmosomes, those areas containing only the Pg and Dp but not PKPs (accessory proteins) as moderately active forming unstable junctions and those areas containing both principal and accessory components as the least active zones expressing stable junctions. Finally, the presence of particular accessory components such as PKP-1 which is normally present in the upper spinous layer of the epidermis indicates an inactive or terminally stabilized zone of cell–cell adhesion.

In the present study, we showed marked variation in protein expression which was associated with the stage of development and the zone being medial or lateral. These variations could be reflection of differences in morphogenic activity. In the cap stage, for example, the dental lamina is strongly labeled for principal and some accessory markers. Before its physiological degradation, the dental lamina shows little if any morphological alteration suggestive of morphogenic activity. In contrast, the enamel organ that is constantly gaining size and exhibiting preferential growth zones also produces stability gradients in cell–cell relationships. The absence of desmosomal markers in the lateral and deep portion of the enamel organ at this stage of development suggests important local morphogenic activity [Figure 3]. It is important to remember that, at these studied stages, the outer and inner enamel epithelial cells remain undifferentiated. The main epithelial cell masses that express variably the adhesion markers are the stellate reticulum cells of enamel organ and the spinous and superficial cells of the oral epithelium, which suppose that this state permits the enamel epithelial cells to undergo the needed morphological and differential processes before transforming into ameloblasts in next late bell stage. This suggests a possible role of desmosomal molecules in tooth morphogenesis, differentiation in a hierarchical and asymmetrical way.

**Conclusion**

In conclusion, our results provide evidence that desmosomal protein expression is highly regulated in developing oral epithelium and enamel organ. In the absence of principal adhesion molecules, no development of desmosomes will occur and active morphogenic events can take place. In the presence of principal desmosomal molecules only, desmosomes are unstable, which is permissive for slow cell rearrangements. Finally, the expression of accessory markers stabilizes the junctions and thus the structural integrity of the epithelial organ is maintained. The fact that a mirror image of protein expression pattern exists between the left and right tooth germs provides further and innovative evidence implicating these structural proteins in tooth morphogenesis.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Keränen SV, Aberg T, Kettunen P, Thesleff I, Jernvall J. Association of developmental regulatory genes with the development of different molar tooth shapes in two species of rodents. Dev Genes Evol 1998;208:477-86.
2. Jernvall J, Thesleff I. Reiterative signaling and patterning during mammalian tooth morphogenesis. Mech Dev 2000;92:19-29.
3. Thesleff I, Sharpe P. Signalling networks regulating dental development. Mech Dev 1997;67:111-23.
4. Thesleff I, Aberg T. Molecular regulation of tooth development. Bone 1999;25:123-5.
5. McCollum MA, Sharpe PT. Developmental genetics and early hominid craniodental evolution. Bioessays 2001;23:481-93.
6. Schwartz MA, Owaribe K, Kartenbeck J, Franke WW. Desmosomes and hemidesmosomes: Constitutive molecular components. Annu Rev Cell Biol 1990;6:461-91.
7. Garrod DR. Desmosomes and hemidesmosomes. Curr Opin Cell Biol 1993;5:30-40.
8. Green KJ, Jones JC. Desmosomes and hemidesmosomes: Structure and function of molecular components. FASEB J 1996;10:871-81.
9. Kelly DE. Fine structure of desmosomes, hemidesmosomes, and an adependimeral globular layer in developing newt epidermis. J Cell Biol 1966;28:51-72.
10. Goodwin L, Hill JE, Raynor K, Raszi L, Manabe M, Cowin P, et al. Desmoglein shows extensive homology to the cadherin family of cell adhesion molecules. Biochem Biophys Res Commun 1990;173:1224-30.
11. Buxton RS, Cowin P, Franke WW, Garrod DR, Green KJ, King IA, et al. Nomenclature of the desmosomal cadherins. J Cell Biol 1993;121:481-3.
12. Cowin P, Mattey D, Garrod D. Identification of desmosomal surface components (desmocollins) and inhibition of desmosome formation by specific Fab'. J Cell Sci 1984;70:41-60.
13. Amagai M, Karpati S, Prussick R, Klaus-Kovtun V, Stanley JR. Autoantibodies against the amino-terminal cadherin-like binding domain of pemphigus vulgaris antigen are pathogenic. J Clin Invest 1992;90:919-26.
14. Stappenbeck TS, Green KJ. The desmoplakin carboxyl terminus coaligns with and specifically disrupts intermediate filament networks when expressed in cultured cells. J Cell Biol 1992;116:1197-209.
15. Bornslaeger EA, Corcoran CM, Stappenbeck TS, Green KJ. Breaking the connection: Displacement of the desmosomal plaque protein desmoplakin from cell–cell interfaces disrupts anchorage of intermediate filament bundles and alters intercellular junction assembly. J Cell Biol 1996;134:985-1001.
16. Cowin P, Kapprell HP, Franke WW, Tamkun J, Hynes RO. Plakoglobin: A protein common to different kinds of intercellular adhering junctions. Cell 1986;46:1063-73.
17. Mathur M, Goodwin L, Cowin P. Interactions of the cytoplasmic domain of the desmosomal cadherin ds1g1 with plakoglobin. J Biol Chem 1994;269:14075-80.
18. Troyanovsky SM, Troyanovsky RB, Eshkind LG, Leube RE, Franke WW. Identification of amino acid sequence motifs in desmocollin, a desmosomal glycoprotein, that are required for plakoglobin binding and plaque formation. Proc Natl Acad Sci U S A 1994;91:10790-4.
19. Wahl JK, Sacco PA, McNamara-Sadler TM, Saupell LM, Wheelock MJ, Johnson KR, et al. Plakoglobin domains that define its association with the desmosomal cadherins and the classical cadherins: Identification of unique and shared domains. J Cell Sci 1996;109 (Pt 5):1143-54.
20. Shabana AH, Amar L, Oboeuf M, Martin N, Forest N. Cytoplasmic desmosome formation by H-7 and EGF treatment in cultured fetal rat keratinocytes. Tissue Cell 1996;28:537-45.
21. Amar LS, Shabana al-HM, Oboeuf M, Martin N, Forest N. Desmosomes are regulated by protein kinase C in primary rat epithelial cells. Cell Adhes Commun 1998;5:1-2.
22. Wallis S, Lloyd S, Wise I, Ireland G, Fleming TP, Garrod D, et al. The alpha isoform of protein kinase C is involved in signaling the response of desmosomes to wounding in cultured epithelial cells. Mol Biol Cell 2000;11:1077-92.

23. Moll I, Houdek P, Schäfer S, Nuber U, Moll R. Diversity of desmosomal proteins in regenerating epidermis: Immunohistochemical study using a human skin organ culture model. Arch Dermatol Res 1999;291:437-46.

24. Alroy J, Pauli BU, Weinstein RS. Correlation between numbers of desmosomes and the aggressiveness of transitional cell carcinoma in human urinary bladder. Cancer 1981;47:104-12.

25. Moll I, Kurzen H, Langbein L, Franke WW. The distribution of the desmosomal protein, plakophilin 1, in human skin and skin tumors. J Invest Dermatol 1997;108:139-46.

26. Krunic AL, Garrod DR, Madani S, Buchanan MD, Clark RE. Immunohistochemical staining for desmogleins 1 and 2 in keratinocytic neoplasms with squamous phenotype: Actinic keratoses, keratoacanthoma and squamous cell carcinoma of the skin. Br J Cancer 1998;77:1275-9.

27. Depondt J, Shabana AH, Sawaf H, Gehanno P, Forest N. Cytokeratin alterations as diagnostic and prognostic markers of oral and pharyngeal carcinomas. A prospective study. Eur J Oral Sci 1998;106 Suppl 1:137-42.

28. Lewis JE, Jensen PJ, Wheelock MJ. Cadherin function is required for human keratinocytes to assemble desmosomes and stratify in response to calcium. J Invest Dermatol 1994;102:870-7.

29. Inagaki M, Irie K, Ishizaki H, Tanaka-Okamoto M, Morimoto K, Inoue E, et al. Roles of cell-adhesion molecules nectin 1 and nectin 3 in ciliary body development. Development 2005;132:1525-37.

30. Obara N, Lesot H. Subcellular localization of beta-catenin and cadherin expression in the cap-stage enamel organ of the mouse molar. Histochem Cell Biol 2004;121:351-8.

31. Bartlett JD, Smith CE. Modulation of cell-cell junctional complexes by matrix metalloproteinases. J Dent Res 2013;92:10-7.

32. Nanci A. Enamel formation and structure. In: Nanci A, editor. Ten Cate's Oral Histology: Development, Structure, and Function. St. Louis: C.V. Mosby; 2003.

33. Mahoney MG, Sadowski S, Brennan D, Pikander P, Saukkko P, Wahl J, et al. Compound heterozygous desmoplakin mutations result in a phenotype with a combination of myocardial, skin, hair, and enamel abnormalities. J Invest Dermatol 2010;130:968-78.

34. Fausser JL, Schlepp O, Aberdam D, Meneguzzi G, Ruch JV, Lesot H, et al. Localization of antigens associated with adherens junctions, desmosomes, and hemidesmosomes during murine molar morphogenesis. Differentiation 1998;63:1-1.

35. Mahoney MG, Sadowski S, Brennan D, Pikander P, Saukkko P, Wahl J, et al. Compound heterozygous desmoplakin mutations result in a phenotype with a combination of myocardial, skin, hair, and enamel abnormalities. J Invest Dermatol 2010;130:968-78.

36. Bartlett JD, Smith CE. Modulation of cell-cell junctional complexes by matrix metalloproteinases. J Dent Res 2013;92:10-7.

37. Inagaki M, Irie K, Ishizaki H, Tanaka-Okamoto M, Morimoto K, Inoue E, et al. Roles of cell-adhesion molecules nectin 1 and nectin 3 in ciliary body development. Development 2005;132:1525-37.

38. Barron MJ, Brookes SJ, Draper CE, Garrod D, Kirkham J, Shore RC, et al. The cell adhesion molecule nectin-1 is critical for normal enamel formation in mice. Hum Mol Genet 2008;17:3509-20.

39. Yoshida T, Miyoshi J, Takai Y, Thesleff I. Cooperation of nectin-1 and nectin-3 is required for normal ameloblast function and crown shape development in mouse teeth. Dev Dyn 2010;239:2558-69.

40. Caterina JJ, Skobe Z, Shi J, Ding Y, Simmer JP, Birkedal-Hansen H, et al. Enamelysin (matrix metalloproteinase 20)-deficient mice display an amelogenesis imperfecta phenotype. J Biol Chem 2002;277:49598-604.

41. Gallicano GI, Kouklis P, Bauer C, Yin M, Vasioukhin V, Degenstein L, et al. Desmoplakin is required early in development for assembly of desmosomes and enhances its recruitment to desmosomes. Implications for cutaneous disease. J Biol Chem 1999;274:18145-8.

42. Bhakta S, Kadowaki T, Inoue E, et al. Roles of cell-adhesion molecules nectin 1 and nectin 3 in ciliary body development. Development 2005;132:1525-37.

43. Lewis JE, Jensen PJ, Wheelock MJ. Cadherin function is required for human keratinocytes to assemble desmosomes and stratify in response to calcium. J Invest Dermatol 1994;102:870-7.

44. Bartlett JD, Smith CE. Modulation of cell-cell junctional complexes by matrix metalloproteinases. J Dent Res 2013;92:10-7.

45. Gallicano GI, Kouklis P, Bauer C, Yin M, Vasioukhin V, Degenstein L, et al. Desmoplakin is required early in development for assembly of desmosomes and enhances its recruitment to desmosomes. Implications for cutaneous disease. J Biol Chem 1999;274:18145-8.

46. Bhakta S, Kadowaki T, Inoue E, et al. Roles of cell-adhesion molecules nectin 1 and nectin 3 in ciliary body development. Development 2005;132:1525-37.

47. Smith EA, Fuchs E. Defining the interactions between intermediate filaments and desmosomes. J Cell Biol 2004;121:351-8.