Immunohistochemical expression of CD56 in dog (*Canis familiaris*) odontogenesis

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ABSTRACT

Aim: To investigate the expression of CD56 in dog odontogenesis in order to elucidate the expression found in ameloblastomas.

Materials and methods: Immunohistochemical analysis of CD56 expression of developing dog teeth in the bud, cap and bell stages including the remnants of the dental lamina.

Results: Weak CD56 expression was observed in the dental epithelium during the bud stage with intense staining of certain peripheral epithelial cells. Positive staining of epithelial cells was also observed in the cap stage with intense staining of the inner enamel epithelium at this stage. During the bell stage the staining was concentrated on the cervical loop areas. The dental papilla revealed positive staining throughout the cap and bell stages while the dental follicle stained intensely positive throughout all the phases examined. The dental lamina and Serres rests also stained positive for CD56.

Conclusions: The expression of CD56 in dog odontogenic tissue varies according to the stage of tooth development. There is a positive correlation between the positive staining observed in ameloblastomas and their odontogenic cells of origin.

1.   Introduction

CD56 (neural cell adhesion molecule, NCAM) is a transmembrane molecule that belong to the immunoglobulin superfamily.[1] It is a specific marker for natural killer (NK) cells and is also expressed on the surface of various cells including glia and neurones.[2, 3] This molecule mediates homophilic adhesion in certain cell types.[1] Together with other substrate adhesion molecules, CD56 is associated with processes of emigration and early migration of neural crest cells. CD56 expression is retained after neural crest cells have left the neuroectoderm on their way to the pharyngeal arch destinations and labels a subpopulation of neural crest cells in long tailed monkeys (Macaca fascicularis).[4] Physiologically, CD56 functions in the cohesion of epithelia, conditioning of cell migration and transmigration via biological membranes and provide means of homing cells in a new environment.[5] CD56-positive subpopulations of human craniofacial muscle-derived cells have been shown to contain lineage committed myogenic cells as well as multipotent cells that are able to form fat and bone tissue.[6]

CD56 is expressed in a variety of tumours including multiple myeloma,[7] serous neoplasm of the ovary,[8] neuroblastomas[5] and neuroendocrine tumours.[9] The expression thereof is also well documented in ameloblastomas.[10,11] The origin of these odontogenic tumours seems to be the dental lamina and more specifically the remnants thereof referred to as Serres rests.[12] It is therefore crucial to examine the tissue of origin to elucidate the staining patterns observed in the associated tumours. To the best of our knowledge, there are currently four studies where CD56 expression was studied in developing mouse teeth[13-16] while another two studies evaluated the CD56 expression in human impacted third molars.[17, 18] In the latter two cases the tooth crown was already formed and CD56

expression was only investigated in the ectomesenchymal cells of the dental follicle and third molar pad.[17, 18]

It was stated in 2010 that there is still a lack of understanding of the context in which CD56 is expressed in odontogenic epithelium and therefor further investigation is warranted.[10] In this study we therefor aimed to elucidate the expression of CD56 expression in tooth development in order to understand and explain the staining patterns observed in ameloblastomas.

Although most of the studies on tooth development are done on mice and rats one should be careful in extrapolating results as mice do not have canine or premolar teeth.[19] Dogs on the other hand have three incisors, one canine, four premolars and two molars in each maxillary quadrant. The same pattern occurs in the mandibular quadrants with the exception that dogs have a third molar in the mandible.[19] This corresponds with the two incisors, one canine, two premolars and two or three molars in the human dentition.[19]

2.   Materials and Methods

2.1   Tissue acquisition and processing

Twenty four foetuses of large breed dogs were obtained under ethical clearance of the Animal Use and Care Committee of the Faculty of Veterinary Sciences, University of Pretoria, South Africa, from female dogs scheduled for elective pregnancy termination. The foetuses were fixed in 10% buffered formalin and the heads carefully cut into coronal sections, dehydrated and embedded in paraffin wax blocks. Those samples that contained calcified bone or dental hard tissues were decalcified in routine decalcifying solution (70ml

HNO_3, 50ml HCL, 880ml distilled water) for 60 minutes and then rinsed in running tap water for 60 minutes before processing. Tissue specimens were sectioned at 3µm, stained with haematoxylin and eosin (H&E), and microscopically examined to select slides with well-formed enamel organs in which the respective odontogenic structures were morphologically clearly identifiable.

Although tooth development occurs as a continuous process, we divided the process into the bud, cap and bell stages in order to differentiate between variations in the staining pattern. The following criteria were used to determine the relevant stage of tooth development. The bud stage was defined as the stage where epithelial cells proliferated into the underlying ectomesenchyme without formation of a cap shape or presence of stellate reticulum (SR).   A tooth germ was regarded as being in the cap stage when the enamel organ resembled a cap on a ball of ectomesenchyme and SR cells were observed without the presence of differentiated ameloblasts or odontoblasts. The bell stage was characterized by the advanced features where differentiated ameloblasts and odontoblasts were visible with or without the presence of hard tissue formation. The cervical loop area was defined as the area where the inner enamel epithelium (IEE) and outer enamel epithelium (OEE) meet. The dental lamina breaks up during the bell stage and therefor Serres rests were examined on these slides.

2.2   Immunohistochemical staining

Immunohistochemical staining was performed on 3 µm sections cut from formalin fixed, paraffin embedded tissue blocks using antibodies to CD56 (clone 123C3, 1:50 dilution,

DAKO, Carpinteria, CA). Staining was performed on the Ventana Benchmark GX automated system (Ventana Medical Systems, Arizona USA). The protocol of 80 minutes antigen retrieval in CC1 (Ventana), two hours of titrated antibody incubation and detection with the Optiview DAB IHC kit (Ventana) was followed. Controls were included for positive and negative staining. The sections were dehydrated, cleared, mounted with a permanent mounting media and evaluated with a light microscope. Brown cytoplasmic and/or membrane staining was interpreted as positive for the presence of CD56.

3.   Results

CD56 staining patterns are summarized in table 1. During the bud stage, the odontogenic epithelial cells in general revealed weak positive staining for CD56 with intense positivity in a large portion of the palisaded peripheral cells (Fig.1). Strong and diffuse positivity was also observed in the condensed ectomesenchyme surrounding it. Throughout the cap stage the OEE and SR stained positively for CD56 while intense positivity was observed in the peripheral aspects of the IEE (Fig.2). The central aspect of the IEE, associated with the enamel knot, revealed only weak staining. All through this stage weak staining was observed in the dental papilla while the dental follicle stained intensely positive for CD56. For the period of the bell stage, CD56 staining of the enamel organ seemed to become concentrated at the cervical loops revealing intense positivity of the IEE, stratum intermedium (SI), SR and OEE in this area (Fig.3). Differentiated IEE cells/ameloblasts located more towards the coronal aspect were devoid of any staining. For the duration of this stage the dental pulp and follicle maintained its CD56 expression. On the sections
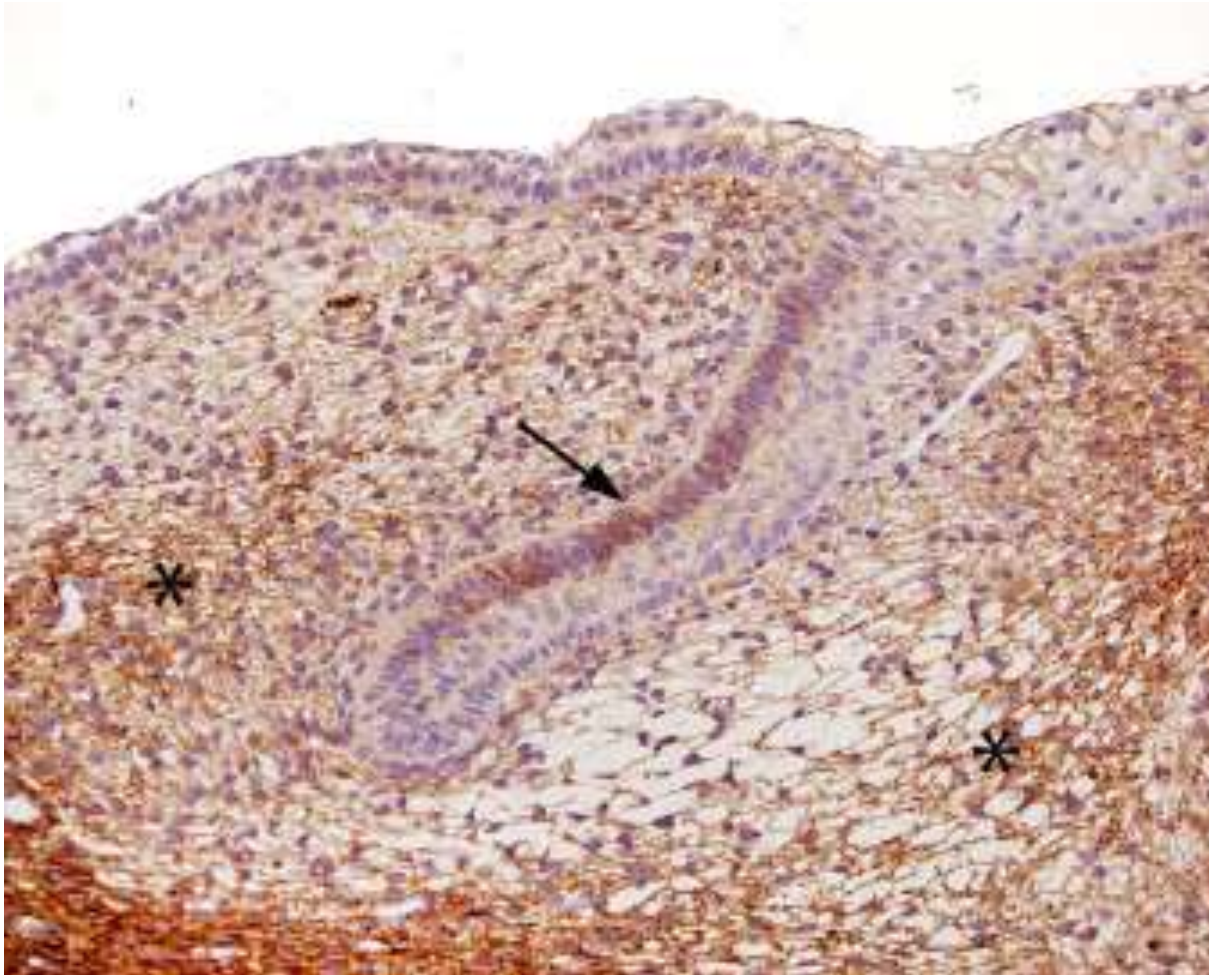
6

Fig. 1 – Intense CD56 staining of the majority of the palisaded peripheral odontogenic epithelial cells (arrow) in the bud stage with diffuse positivity of the associated condensed ectomesenchyme (asterisks) (original magnification X100).
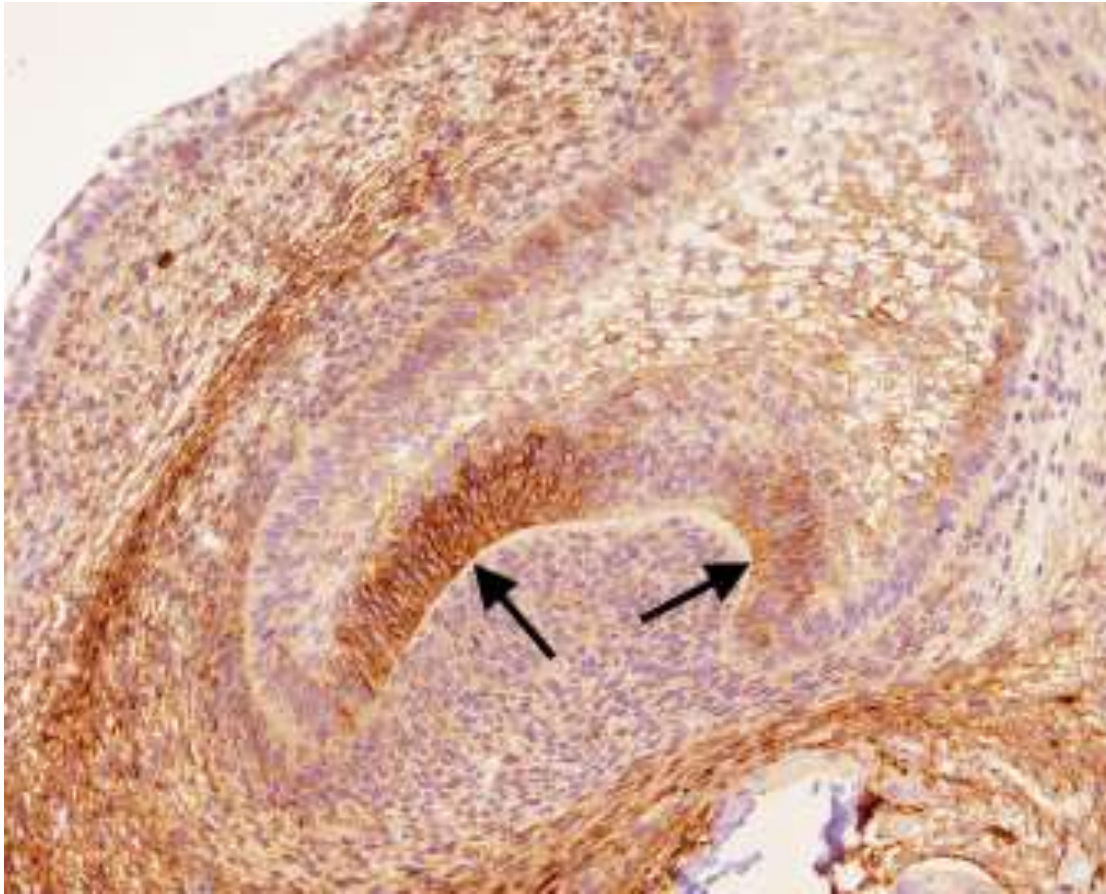
Fig. 2 – Positive staining of the OEE and SR with intense positivity of the IEE (arrow, excluding the central area associated with the enamel knot). Weak staining of the dental papilla with intense CD56 positivity in the dental follicle (original magnification X100).
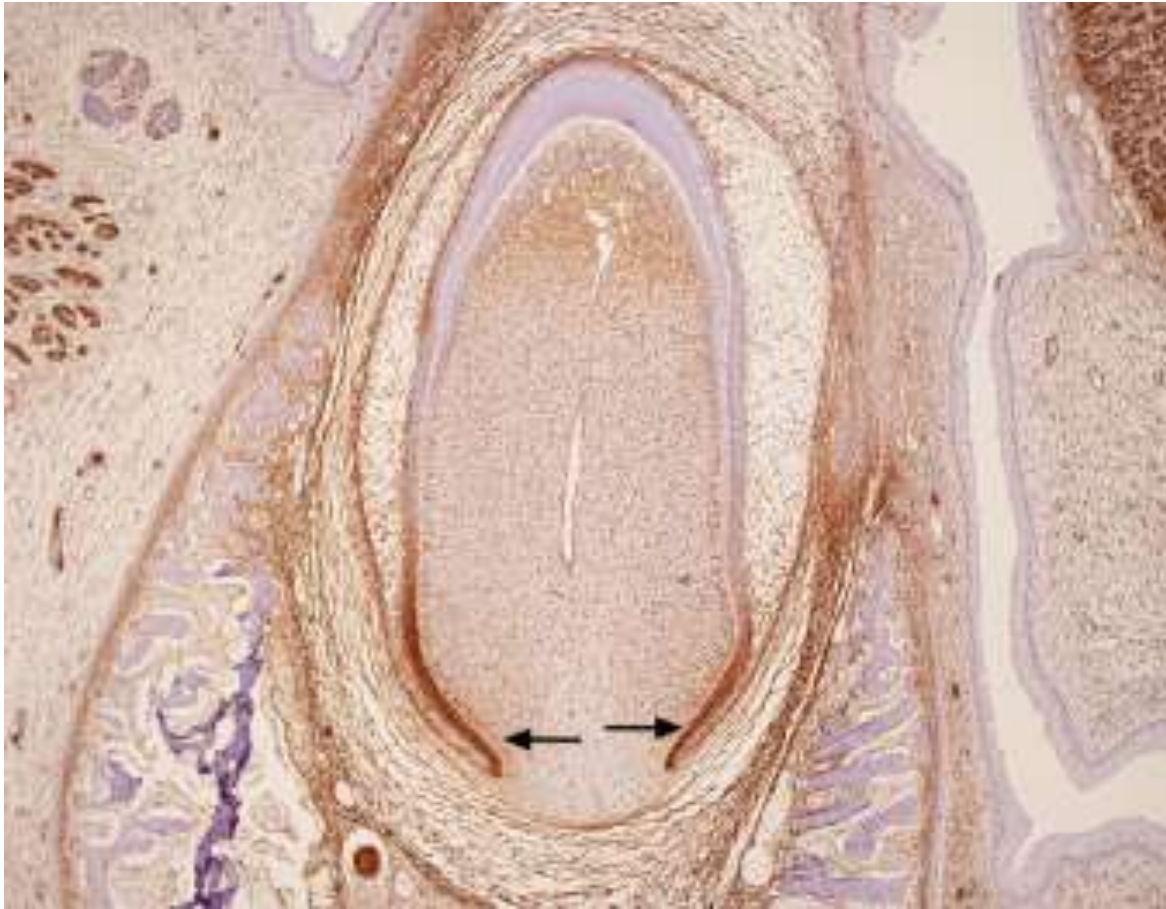
Fig. 3 – CD56 staining of the enamel organ in the bell stage. Intense positivity at the cervical loop areas including the IEE (arrows), SI, SR and OEE. The dental pulp and follicle also reveal positive staining for CD56.

Developmental stage	Location	Weak staining	Moderate staining	Intense staining	No staining
Table 1 - CD56 staining patterns observed in the respective stages of tooth development.					
Bud	Odontogenic epithelium	19/19			
n=19	Palisaded peripheral odontogenic epithelial cells		13/19	6/19	
	Condensed ectomesenchyme			19/19	
Cap	OEE and SR	5/12	7/12		
n=12	Central IEE	12/12			
	Peripheral IEE		5/12	7/12	
	Dental papilla	12/12			
	Dental follicle			12/12	
Bell	Differentiated ameloblasts				16/16
n=16	IEE, OEE and SR associated with cervical loop			16/16	
	Dental papilla		16/16		
	Dental follicle			16/16	
	Dental lamina		11/14*	3/14*	
	Serres rests		11/14*	3/14*	

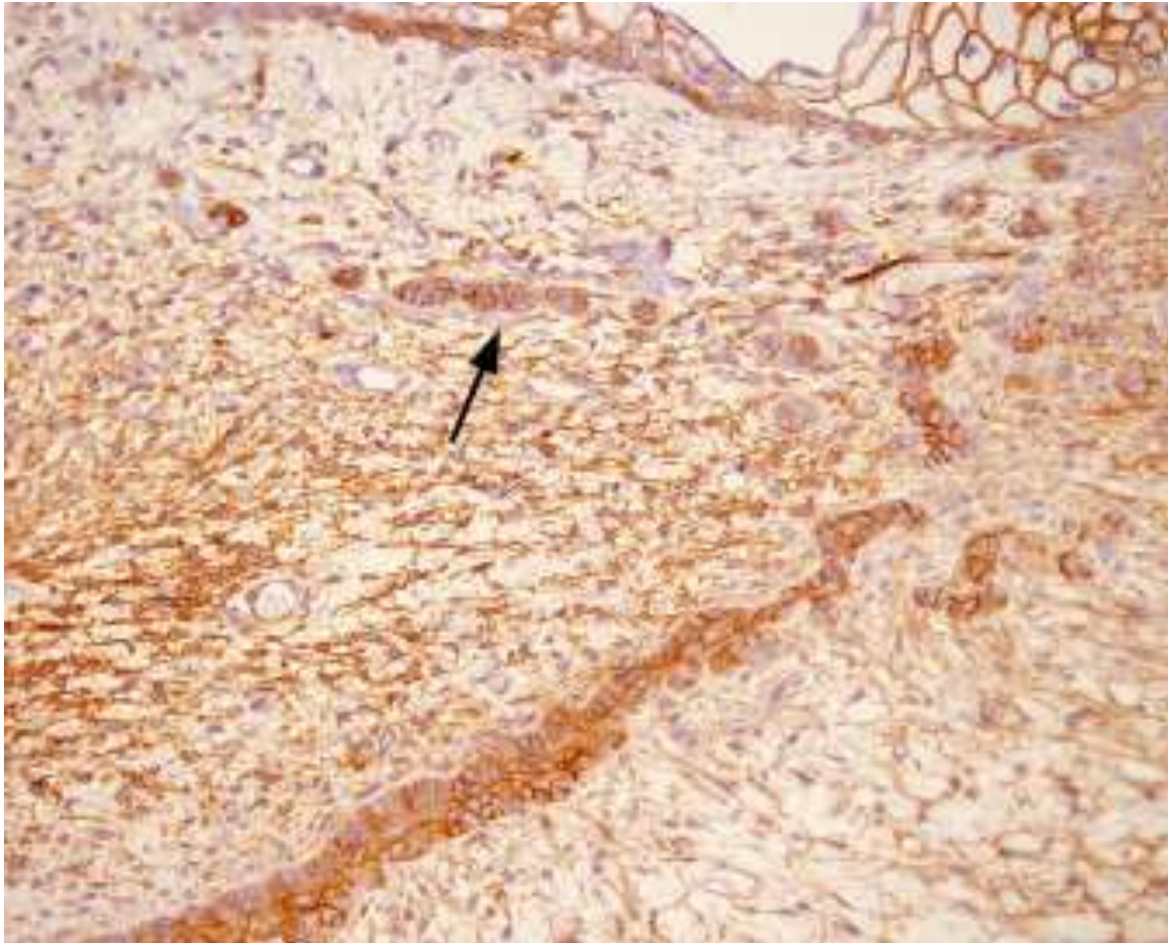* Dental laminae and Serres rests were identified in 14 of the bell stage sections.

Fig. **4** – CD56 positive staining of the dental lamina and Serres rests (arrow).

where the dental lamina and Serres rests were clearly identifiable, these structures

constantly revealed CD56 positivity (Fig.4).

4.   Discussion

The positive staining of the epithelial cells during the bud stage was more pronounced in the

peripheral cells especially those in a more advanced stage of development. This is in

contrasts with the findings of Obara *et al.* where the inner cells of the bud stage epithelium

stained positive for CD56.[15]

The staining pattern observed in the cap stage is in agreement with the findings of

Obara *et al.* as intense staining of the IEE adjacent to the enamel knot area corresponds to

the "inner part of the enamel organ in a limited area close to the outer enamel epithelium".

The positive staining of the papilla and follicle is also in concert with the staining patterns

found in mice. [15]

Obara *et al.* described the enamel organ cells as mostly negative in the early and late

bell stages with the exception of transient positivity in the SI.   This is inconsistent with our

findings as a constant pattern of intense positivity was observed in the cervical loop are

including IEE, SI, SR and OEE. Although Obara *et al.* only detected transient staining in the

cuspal aspect of the dental papilla at the early bell stage*,* the dental pulp revealed constant

positivity associated with the cervical loop areas in all cases of the present study. The dental

follicle however revealed strong positive staining throughout the experimental period in

both studies.[15]

It has been speculated that CD56 expression pattern should be similar in human and murine tooth germs,[12] but one should be careful in extrapolating results between species as mentioned earlier and supported by the results obtained in this study. The CD56 expression in mouse incisors have been shown to differ from that found in molar teeth, most probably as a result of the continuous eruption of mouse incisors compared to the limited eruption of molars.[13] Another possible reason for the differences observed, could be the fact that CD56 is modulated by alternative RNA splicing, resulting in different cytoplasmic domains that differentially interact with the cell membrane. This regulatory mechanism may link CD56 binding function with other primary cell processes during embryonic development.[1]

CD56 distribution has been proven to exhibit dynamic changes during mouse molar development.[16] Although CD56 is expressed in neural cells including Schwann cells, the pattern of expression thereof in the dental follicle differs from the pattern of innervation as revealed by a study correlating the anti-NF antibody expression.[16] Loss of CD56 has been proposed to correlate with the differentiation of follicular cells.[16] This is in agreement with the current results, as the IEE cells lost their CD56 expression as they differentiated into ameloblasts. Therefor CD56 may be involved in retaining the structure of the dental organ and regulate cell-cell interaction during odontogenesis.[16]

Various non-neuronal cells express CD56 during embryonic development and some of them (lung epithelium and dermal papilla of feathers) even express this molecule in adult life.[20, 21] It has been proposed that persistent CD56 expression in adult life reflects the retained potential of differentiation and morphogenesis even after development has ceased.[16] Persistent expression of CD56 in the dental pulp have been proposed to correlate with the regenerative potential of the dental pulp as the CD56 expression may have

marked undifferentiated ectomesenchymal cells.[16] Another study on the dental follicle of impacted human third molars proposed that CD56 staining identified immature fibroblast cells with phenotypic features of stromal cells in the dental follicle and the authors agreed that CD56 may be used as a stem-cell marker as well as a marker for stromal phenotype.[17]

Even though the exact cells of origin of ameloblastomas is still unknown,[22] the dental epithelial cells including the dental lamina and enamel organ cells seem to be responsible for these tumours.[23] The remnants of the dental lamina, Serres rests, are therefor role players in the pathogenesis of ameloblastomas. In this study the dental lamina and Serres rests revealed positive staining for CD56 throughout the investigation. This correlates with the expression of CD56 in ameloblastomas. In 2001 Er *et al.* described CD56 staining in the peripheral cells of follicular ameloblastoma tumor nests, with absence in the central stellate reticulum cells.[11] This corresponds with the staining patterns observed in our study during the bud, cap and bell stage where certain cells stained intensely positive while others were devoid of CD56 staining. The proposal that the outer columnar cells of ameloblastomas may transdifferentiate to neuroectodermal cells should also be kept in mind.[12]

In conclusion, CD56 expression in odontogenesis is spatially and temporally regulated resulting in heterogeneous expression patterns during the different stages of tooth development. It is positively expressed in the dental lamina and Serres rests which correlate with the expression thereof found in ameloblastomas. Keeping the physiological functions of cell adhesion molecules in mind, CD56 might play a crucial role in tumour biology. It may be implicated in alterations in the cell membrane environment which in turn could increase the local aggressive growth potential of ameloblastomas. However the distinct manner remains to be resolved.

Acknowledgments

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