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Original

Differential Distribution of Type IV Collagen α1 to α6 Chains Suggests Distinct Molecular Interaction between the Epithelial and Mesenchymal Components of Benign Odontogenic Tumors

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Abstract:Type IV collagen is the principal constituent component of the basement membrane(BM). It have been reported that the alpha chains of type IV collagen [α(IV)] showed temporal and spatial localization pattern in different stages of the developing tooth germ except α3(IV) chain. In this study, we investigated the localization of α(IV) chains in benign odontogenic tumor in comparison with their localization in the cap stage human tooth germ by immunohistochemistry.
All the tumor samples studied as well as the tooth germ were negative to α3(IV). In ameloblastoma that resembles the enamel organ of the tooth germ, all types of tumor (follicular, plexiform and desmoplastic) were not reactive to α4(IV) chains similar to the α(IV) chains composition in the BM of outer enamel epithelium and dental lamina. All α(IV) chains except α3(IV) were localized in the tumors in which the odontogenic epithelium is accompanied by odontogenic ectomesenchyme like adenomatoid odontogenic tumor (pseudoglandular spaces), odontoma and ameloblastic fibroma and this is similar to the α(IV) chains composition in the BM of inner enamel epithelium of the tooth germ. From the results, the presence of α4(IV) chains is thought to be important for the differentiation and induction of the dental papilla like tissue formation in odontogenic tumors.

Key words: Benign odontogenic tumors, Human tooth germ, Immunohistochemistry, Type IV collagen

Introduction

Odontogenic tumors are neoplasms arising form the various tooth forming apparatus. In the latest WHO Histological Classification of Odontogenic Tumors [1], odontogenic neoplasms are first classified as either malignant or benign. The malignant tumors are then categorized as carcinomas and sarcomas depending on whether the malignancy is of either odontogenic epithelial or ectomesenchymal compartment. Likewise, the benign tumors are classified, whether they are odontogenic epithelial or ectomesenchymal in origin, the former with or without odontogenic ectomesenchyme. The presence or absence of inductive dental hard tissue formation is also considered. Except amelobalstoma, which is locally invasive, majority of odontogenic lesions are either benign or harmatomatous malformation (e.g., odontomas) [2-4]. The occurrence of malignant odontogenic neoplasms, such as odontogenic carcinoma and odontogenic sarcoma is very rare [5,6]. The diverse biological characters of different odontogenic tumors with wide histomorphologic spectrum underline the necessity for better understanding of the molecular mechanism involving the formation and development of these lesions in comparison with the differentiation and development of the tooth germ.

Odontogenesis is a complex process, which needs inductive and reciprocal interaction between the odontogenic epithelium and the ectomesenchyme in order to control cell differentiation and morphogenesis [7,8,9]. Basement membrane (BM), a thin, sheet like, highly specialized structure of extracellular matrix, interposed between the two tissues plays a pivotal role during the epithelial-mesenchymal interactions [8,10,11]. It is crystal clear that the growth factors and cytokines secreted from the dental epithelium and ectomesenchyme interact mutually through the constituent components of the BM [12]. The BM is composed of a number of extracellular matrix molecules primarily of type IV collagen,

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nidogen, laminin and heparan sulphate proteoglycan[13]. With improvement in the knowledge of BM composition and biology, the molecular structure of BM is even more complex and become evident that individual proteins in BM act as modulators of specific biological functions such as cellular growth, differentiation, repair, migration not only in the normal developmental processes but also in tumor cell differentiation, invasion and metastasis of different pathological events [13,14,15,16].

Earlier studies have examined various BM constituents including collagen IV and laminin to determine their roles during tooth morphogenesis and cytodifferentiation [9,17]. The binding of type IV collagen in the basement membrane matrix with Transforming Growth Factor beta-1(TGFβ-1) demonstrated that extracellular matrix components may function as an affinity matrix for binding and immobilizing soluble growth and differentiation factors and imply in local regulation of cell proliferation and differentiation [18]. This finding draws special attention to the role of different type of collagen molecules in the histodifferentiation of the tooth germ but there was no significant change in their immunolocalization with the stages of tooth germ development [19].

Type IV collagen, the major component of the mammalian BM assembly, can be further divided into six distinct poly peptide chains designated as α1(IV) to α6(IV) and are encoded by six distinct genes, *COL4A1* to *COL4A6* [20,21,22,23]. The self-association of type IV collagen triple-helical molecules, composed of three α chains, forms the supramolecular network of the BM. At least three different compositions of type IV collagen triple helix have been identified in human with their tissue specific distributions[24]. $[\alpha1(IV)]_2$ α2(IV) is ubiquitously distributed in all BM while α3(IV), α4(IV) and α5(IV) is abundant in lung alveolar and glomerular BM and α5(IV)/α6(IV) is localized in the BM of mammary duct and lobule, epidermis, prostate gland and smooth muscle cells [13,14,25,26].

Nagai et al. have reported that collagen IV isoforms localized in the dental BM showed stage and position specific distribution patterns at various stages of mouse molar germ development [27]. Differential expression of α(IV) chains in BMs of benign and malignant odontogenic tumors have been studied and the authors suggested that modification and remodeling of these molecules occur during odontogenic tumor progression [28,29].

The objective of this article is to compare the localization and distribution of type IV collagen a chain isoforms in human tooth germ to that in benign odontogenic tumors in order to get an insight evidence for molecular classification and cellular differentiation of the tumor components.

Materials and Methods

Archival formalin-fixed, paraffin embedded tissue blocks of eight ameloblastomas (five ordinary and three desmoplastic), two adenomatoid odontogenic tumors, three ameloblastic fibromas and three odontomas were retrieved from the surgical pathology unit of the Department of Oral Pathology and Medicine, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Japan. Embryonic human tooth germ (in the cap/early bell stage) was kindly provided by Professor Shigehisa Yamamoto from the Department of Oral Histology, Ohu University Dental School.

4μm sections were prepared for routine staining with hematoxylin-eosin and also for immunohistochemistry. Histological diagnoses of these tumor entities were made in accordance with the WHO histological classification of odontogenic tumors [1] and other established criteria in current use.

Monoclonal antibodies

Rat monoclonal antibodies, H11, H22, H43, M54 and M69 recognizing type IV collagen α1, α2, α3, α4, α5 and α6 chains, respectively, were raised against synthetic peptides of nonconsensus amino acid sequences of the human alpha (IV) chains. The epitopes were determined by multipin-peptide scanning and the specificity of the antibody against the individual human α(IV) chains was confirmed by enzyme-linked immunosorbent assay (ELISA) and immunoblotting.

Immunohistochemistry

For immunohistochemical staining, deparaffinized and rehydrated sections were immersed in methanol containing 0.3% hydrogen peroxide for 30 min to block endogenous peroxidase. These sections were then pretreated for antigen retrieval by autoclave heating (132°C, 3 min) in 10nM citrate buffer (pH 3.3), and rinsed in 0.05 M Tris-buffered saline (TBS) for 5 min, three times before immersing the sections in blocking solution (Funakoshi, Japan) for 10 min at room temperature. Thereafter, the sections were covered with the optimal dilutions of primary antibodies and incubated overnight at 4°C (α1(IV) and α2(IV) 1:400,α3(IV) 1:100,α4(IV) 1:10, α5(IV) 1:100 and α6(IV) 1:20). The immunoperoxidase reaction was performed using Vectastain Elite ABC kit (Vector, Burlingame, Calif., USA). The antigenic sites were demonstrated by reacting sections with a mixture of 0.05% 3,3'-diaminogenzidine tetrahydrochloride (Vector) in 0.05 M of Tris-HCL buffer (pH 7.6) containing 0.01% H_2O_2 for 7 min. The nuclei were counterstained with hematoxylin. For negative control, sections were reacted with normal rat serum or with the secondary antibody alone. All the control sections were negative. Positive staining controls were included for each antibody and where present in the specimens, internal staining controls were checked for appropriate reactions with each antibody.

Results

The distribution patterns ofα(IV) chains in the BMs of human tooth germ and benign odontogenic tumors were detailed below. α3(IV) chains were not detected in all the specimens studied and α4(IV) chains were scarcely detected only in certain tumors.

Human tooth germ

The outer enamel epithelium of the tooth germ and dental lamina showed reactivity to α1(IV),α2(IV), α5(IV) and α6(IV) chains while the inner enamel epithelium was also reactive to α4(IV) chains in addition to the former isoforms. Moreover, the staining for α1(IV) and α2(IV) chains were strongly detected in the BMs of blood vessels and also that of buccal mucosa .(Fig. 1, a-f)

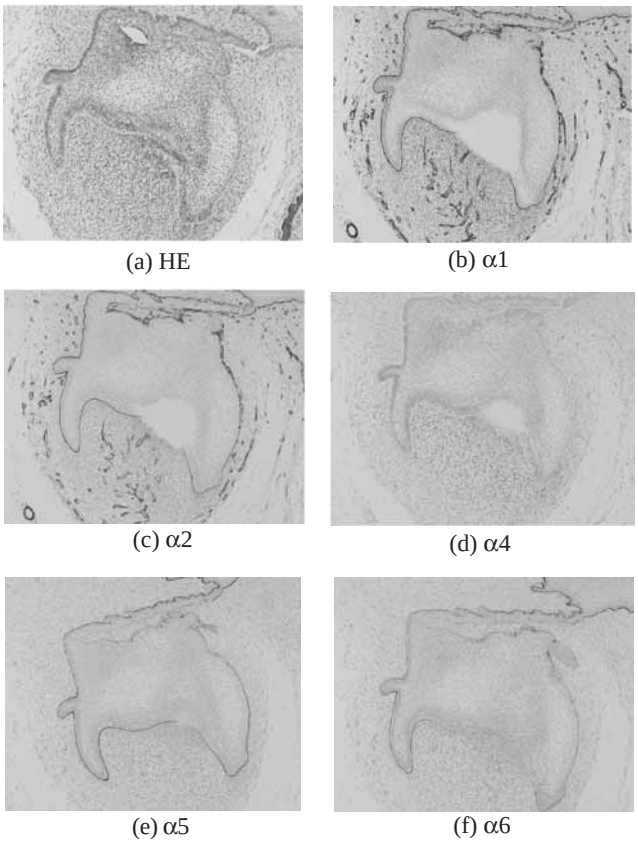

Fig. 1 Type IV collagen alpha chains distributions in cap/early bell stage tooth germ. (a) HE (b-f) α1, α2, α4, α5, α6 chains.
α1 &α2 chains localized in the BMs of the inner and outer enamel epithelium as well as in the BMs of the blood vessels. α5 and α6 chains were localized only in the BMs of the tooth germ but not in the BMs of blood vessels. α4 chains were only localized in the BM of inner enamel epithelium although the staining is weaker than other a chains.

Ameloblastoma

All the ameloblastoma samples studied were negative to α4(IV) chains. Although no significant difference in distribution of α(IV) chains was observed for the different types (follicular, plexiform) and histologic subtypes (granular, acanomatous etc.) among conventional ameloblastomas, distinguishable staining patterns were observed between conventional and desmoplastic types. The immunolocalization of α1(IV) and α2(IV) occurred as thin, intermittent line in the basement membranes encapsulating the epithelial nests (follicular) and strands (plexiform) while α5(IV) and α6(IV) chains were colocalized as continuous linear patterns demarcating the tumor epithelium from the surrounding connective tissue stroma, in conventional ameloblastomas (Fig.2, a-e).

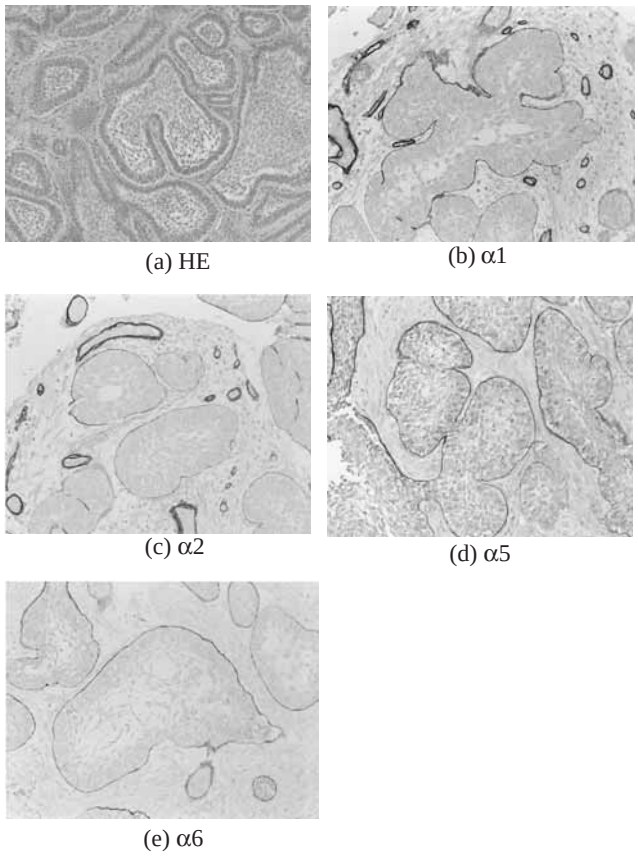

Fig. 2 Type IV collagen alpha chains distributions in follicular ameloblastoma. (a) HE staining (b-e) α1, α2, α5, α6 chains.
In the BMs surrounding the tumor nests, α1 (IV) and α2 (IV) chains were seen as thin, linear pattern with limited areas of discontinuation while α5 (IV) and α6 (IV) chains were seen as continuous linear pattern. The BMs of the blood vessels showed stronger and continuous linear staining to α1 (IV) and α2 (IV) chains compared to the BMs of tumor nests but did not stained with α5 (IV) and α6 (IV) chains.

Random intracellular staining in the tumor islands was also observed for these chains. In desmoplastic type ameloblastomas (Fig.3, a-e), strong linear continuous immunoreaction to all α1(IV), α2(IV), α5(IV) and α6(IV) is evident dividing the neoplastic epithelial nests from the hyalinized collagenous stroma tissue.

Ameloblastic fibroma

All five isoforms of type IV collagen α1(IV), α2(IV), α4(IV), α5(IV) and α6(IV) demonstrated as uniform continuous pattern and compartmentalized the neoplastic epithelial islands and strands from the surrounding dental papilla-like mesenchymal tissue. The five alpha chains also randomly labeled the peripheral pre-ameloblast like cells as well as the central stellate cells. (Fig. 4, a-f)

Adenomatoid odontogenic tumor

Distinct labeling pattern of collagen IV a chains was observed in different areas of AOT. α1(IV), α2(IV), α5(IV) and α6(IV) chains were strongly localized at the boundaries of the cribriform pattern epithelial strands and the hemorrhagic stroma (Fig.5, a-e)

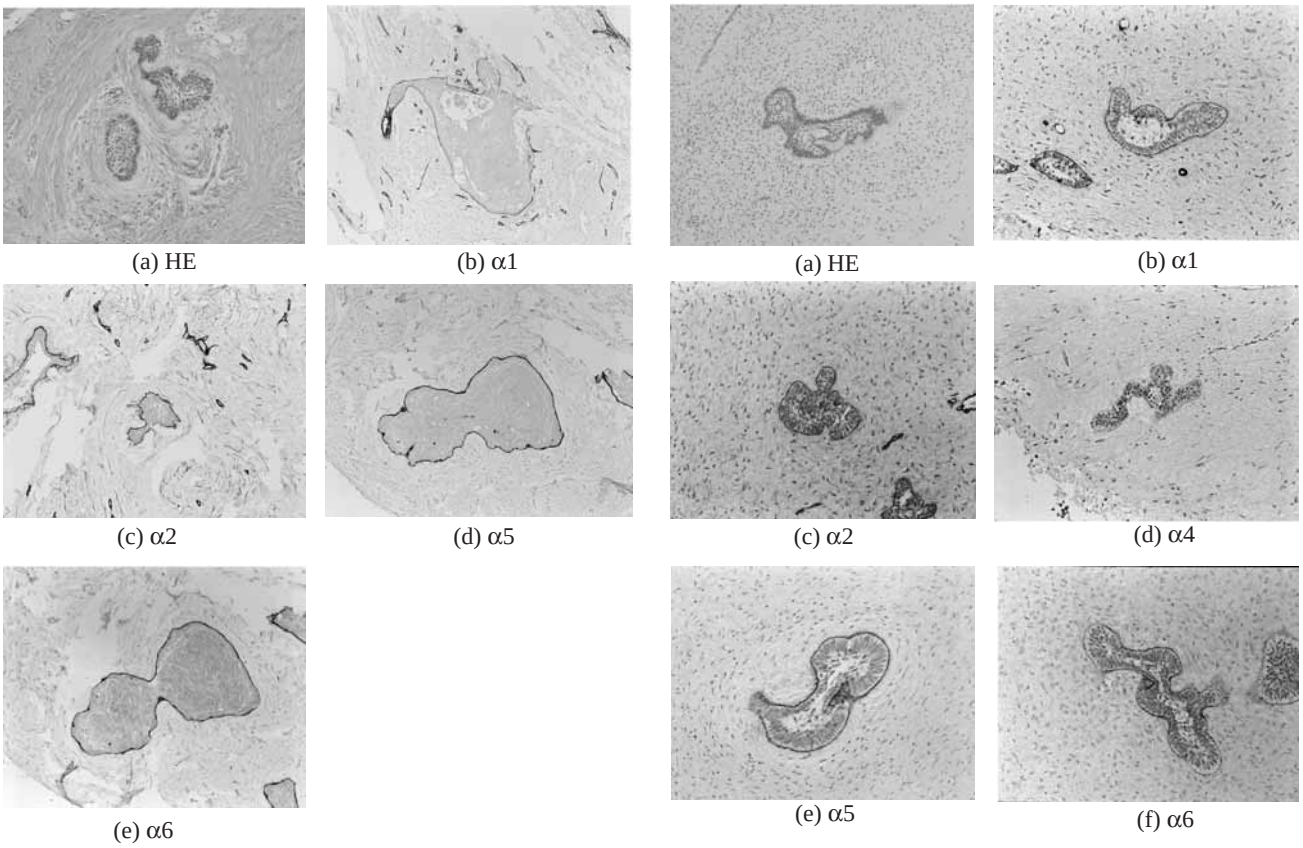

Fig. 3 Type IV collagen alpha chains distributions in desmoplastic ameloblastoma. (a) HE stain (b-e) α1, α2, α5, α6 chains. Generally, the distribution of α(IV) chains was similar to that in conventional ameloblastoma with overall stronger staining intensity. The only difference was continuous α1, α2 chains staining around the tumor nests of desmoplastic ameloblastoma. α5, α6 chains were detected strongly as in conventional type.

Fig. 4 Distribution patterns of collagen IV alpha chains in ameloblastic fibroma. (a) HE staining (b-f) α1, α2, α4, α5, α6 chains.
The small strands and nests of dental epithelium and the dental-papilla-like mesenchyme in ameloblastic fibroma were clearly separated by continuous linear staining to all five alpha chains except α3 chains. Note the strong positivity to α4 chains.

but BMs around the masses of solid epithelial whorls/rosettes and the outer aspect of the duct-like structures only showed faint to negative immunoreactivity. The eosinophilic hyaline droplets located between the opposing rows of columnar cells in convoluted structures of epithelial whorls and in the luminal surfaces of the duct-like structures were also stained positively to α4(IV) in addition to the other four chains (Fig.5, f-k). These amorphous materials showed most intense immunoreactivity to α5(IV) followed by α6(IV), chains (Fig.5, j,k). The mineralized masses and the small epithelial nest with squamoid cells with amyloid-like globules were not reactive to any of α(IV) chains.

Odontoma

The BMs at the interface of the small flat odontogenic epithelium covering the mineralized odontogenic tissue and the underlying mesenchymal tissue containing small flat cells were positive to all fiveα(IV) chains (Fig.6, a-f). In addition, the reactivity to α4(IV) chains was also detected in the pulpal side of the odontoma at the root portion .

Discussion

A number of studies have investigated the characters of the cellular components and the morphological and functional differentiation of tumor cells compare to that of the tooth germ [10, 1, 30, 31, 32]. However, little have known for the participation and molecular composition of the extracellular matrix molecules especially of the basement membrane in tumor histogenesis. Previously, most studies used polyclonal or monoclonal antibody to type IV collagen molecule as a whole and so no significant change in alpha chains composition could be observed among the BMs [10,11,17,33]. Other studies which used the specific monoclonal antibody to α(IV) chains isoform were performed in mouse tooth germ and in odontogenic tumors independently[27,28,29]. In current study, we examined the localization of α(IV) chains in odontogenic tumors using the oral mucosa epithelium and the cap/early bell stage tooth germ of the human embryo as control.

The results of this study demonstrated that collagen α(IV) chains composition and distribution in the BMs of human tooth germ and of different benign odontogenic tumors were distinct. This variation in α chain isoforms suggested having relation with different composition and functional interaction between the epithelial and mesenchymal components of the tooth germ.
Depending on the similarities of the α(IV) chains distribution in

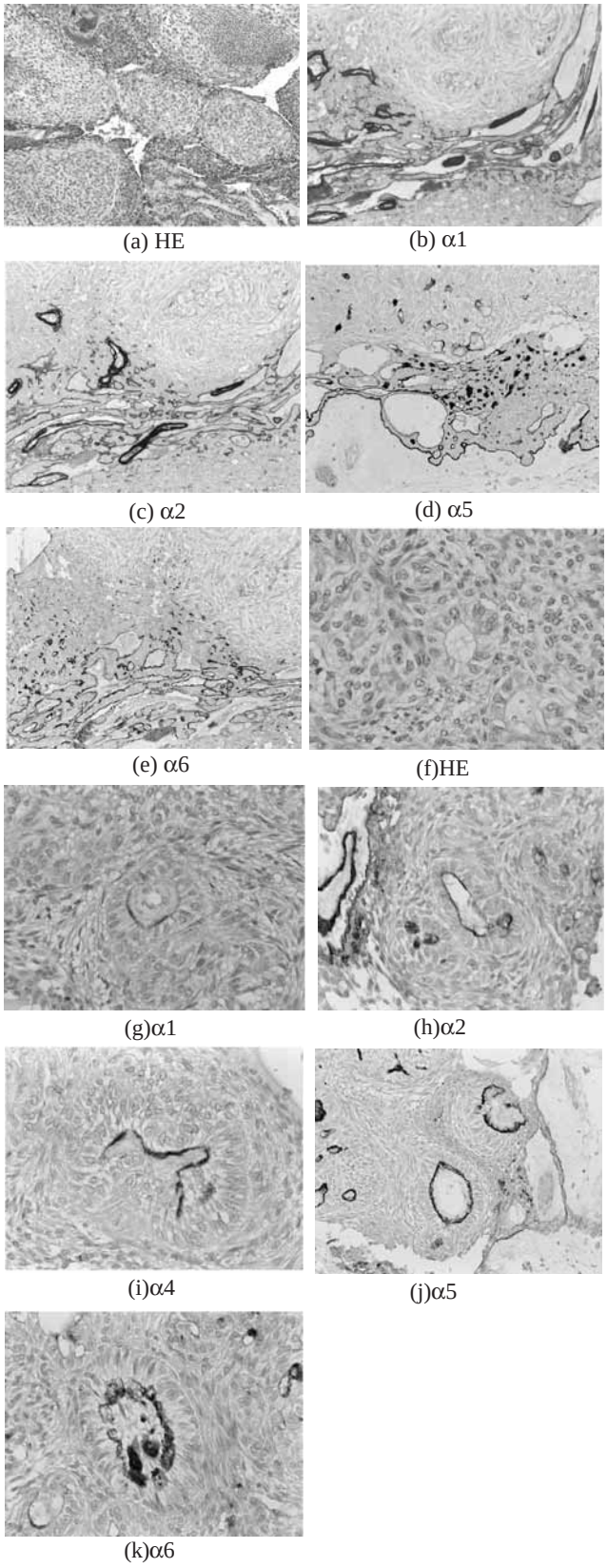

Fig. 5 Distribution of collagen IV alpha chains in adenomatoid odontogenic tumor (AOT), (a,f) HE (b,g) α1 (c,h) α2 (i) α4 (d,j) α5 (e,k) α6 chains. (a-e) cribriform areas (f-k) pseudo-duct like structure. Strong staining to a1, α2, α5, α6 chains was detected in the BMs of cribriform tumor nests. The hyaline materials in the pseudo-duct like structures showed positivity to all five alpha chains including α4.

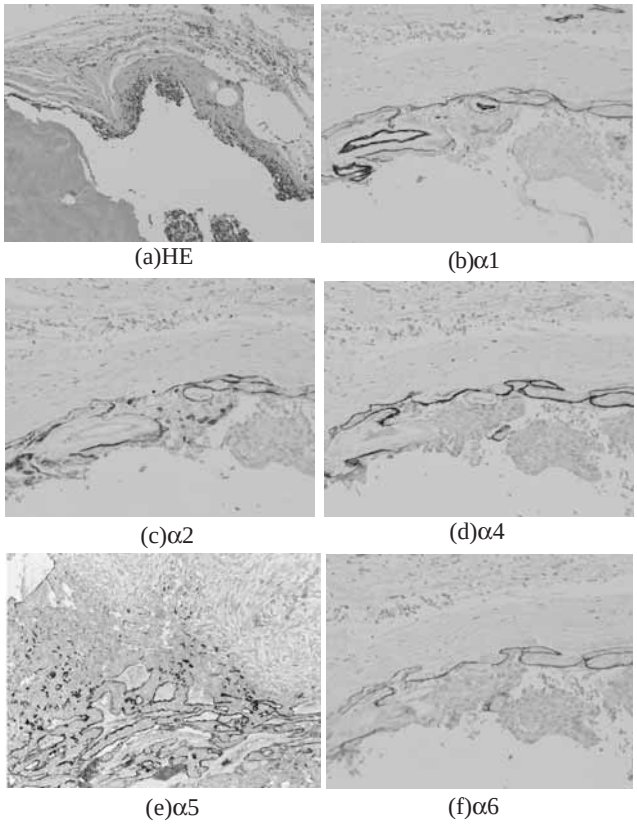

Fig. 6 Distribution patterns of collagen IV alpha chains in odontoma. (a) HE staining (b-f) α1, α2, α4,α5, α6 chains.
The BMs at the interface of the small flat odontogenic epithelium covering the mineralized odontogenic tissue and the underlying mesenchymal tissue containing small flat cells were positive to all five α(IV) chains. chains were also localized in the BM of cervical loop epithelium on the pulpal side

the BMs of the odontogenic neoplasms with that of the human tooth germ, we summarized and divided the examined tumors into two different categories as shown in Fig. 7. Tumors with reactivity to all five alpha chains including α4(IV) chains, similar to the BM of inner enamel epithelium(IEE), are odontoma, adenomatoid odontogenic tumor and ameloblastic fibroma. All types of ameloblastma falls in the negative α4(IV) chains group, similar to the composition of α(IV) chains in the BM of the outer enamel epithelium(OEE) of human tooth germ.

Differences are also noted between the α(IV) chains localization between the human and mouse tooth germ. Although absence of α3(IV) chains reactivity all stages, α1(IV)/ α2(IV) and α4(IV) chains only is detected in the BM of IEE in cap/early bell stage while α5(IV)/ α6(IV) chains immunoreactivity looses from that stage in mouse molar germ [27]. But in cap stage human tooth germ, α1(IV)/ α2(IV) chains and α5(IV)/ α6(IV) chains were detected in all BMs (oral epithelium, OEE) while α4(IV) chains were only observed in the BM between IEE and the dental papilla tissue. So, this finding further support our speculation that α4(IV) chains have putative functional involvement in interaction of odontogenic epithelium and odontogenic ectomesenchyme. We speculated that the absence of α4(IV) chains in BM caused diminished interaction

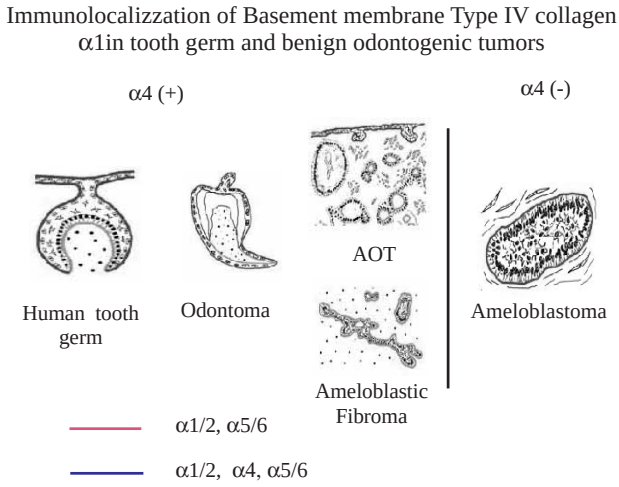

Fig. 7 Schema categorizing the benign odontogenic tumors on the basis of the presence and absence of α4 chains in comparison with the α chains distribution pattern in early bell stage human tooth germ. The red color BMs indicate the localization of α1/α2 and α5/α6 chains whereas the blue color BMs indicate the localization of α1/α2, α4, α5/α6. In human tooth germ, distribution of α1/α2 and α5/α6 chains were seen in the BM of inner enamel epithelium and α1/α2, α4 and α5/α6 chains were seen in the BMs of outer enamel epithelium and dental lamina. Both immunostaining patterns were seen in odontoma and AOT while the staining pattern similar to that of inner enamel epithelium only was seen in ameloblastic fibroma. The α chains staining in the BMs of ameloblastoma tumor nests was similar to that of outer enamel epithelium where α4 chains were not found.

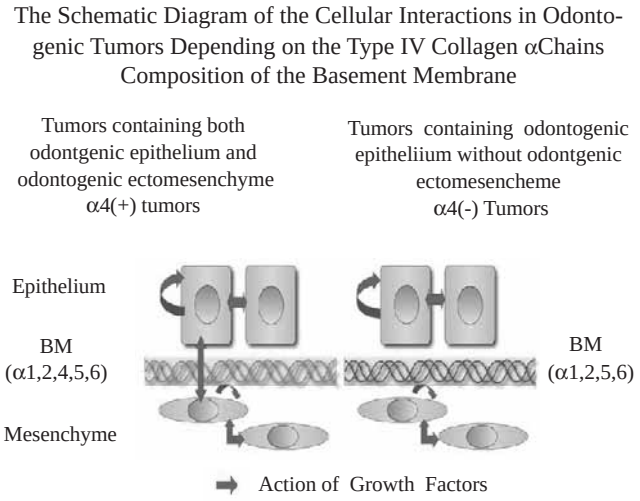

Fig. 8 The speculated mechanisms of α(IV)4 chain positive and negative basement membranes in benign odontogenic tumors.
The presence of α(IV)4 chains in BM of odontogenic tumors may suggest the presence of epithelial-mesenchymal interactions which is important for the differentiation of dental mesenchyme. The epithelial-mesenchymal interaction could not take place in the absence of α(IV)4 chains although there were autocrine and paracrine interactions between homologous cells. However, it was not yet clear that the presence of α(IV)4 chains was whether as the cause or the effect of epithelial-mesenchymal interaction.

between the odontogenic epithelium and odontogenic mesenchyme or vise versa (Fig.8) although the cells maintained the paracrine or autocrine regulation among themselves.

Regarding ameloblastoma, lack of α4(IV) chains pointed out that the epithelial cells in ameloblastoma do not have necessary interaction with the mesenchymal component like epithelial-mesenchymal interaction in the tooth germ from the standpoint of α(IV) chains composition in the BMs. The difference in α1(IV)/ α2(IV) chains staining pattern between conventional and desmoplastic ameloblastomas may be due to their difference biological behavior as demoplastic ameloblastomas are not invasive as conventional ameloblastomas[34]. Other lesions, AOT, odontomas and also ameloblastic fibroma showed continuous linear staining to all five α(IV) chains. So, the limited areas of α1(IV)/ α2(IV) chains discontinuity in conventional ameloblastoma indirectly support the protective role of an intact BM.

As for AOT, although all five α(IV) chains could be localized, α4(IV) chains was only detected in limited areas like in the human tooth germ. This finding favors AOT as a harmatomatous lesion rather then a true neoplasm. We also propose different type of cellular characters in histologically different areas of AOT according to the BM α(IV) chains distribution. It is speculated that the cells forming the cribriform areas are similar to outer enamel epithelium and dental lamina where the BMs lack α4(IV) chains, while the cells forming the whorls and pesudo-duct like structures are similar to preameloblasts or presecretory ameloblasts resting on the BM like in IEE of the tooth germ with occasional production of enamel matrix proteins.

In odontoma and ameloblastic fibroma where odonogenic epithelium as well as odontogenic ectomesenchymal tissue is well-recognized, all α(IV) chains except α3(IV) was observed. The localization was not well-organized like in the human tooth germ but typically very similar. Continuous and strong, linear distribution of α(IV) chains was seen in ameloblastic fibroma where the tumor is mainly composed of dental papilla like ectomesenchymal tissue within which are scatter epithelial islands. Presence of α4(IV) chains in the pulpal side of the root portion of odontoma is particularly interesting and underscore their ostensible importance for differentiation and induction of tooth papilla.

In summary, the alpha chains of BM type IV collagen molecules showed distinct distribution patterns in human tooth germ and in benign odontogenic tumors. α3(IV) chains were totally negative in odontogenic tissue and lesions. According to our results, among the five α(IV) chains localized, α4(IV) chains may have special importance during the interaction of dental papilla with odontogenic epithelium for functional differentiation. Further studies are needed to clarify, the differential distribution of type IV collagen alpha chains in the tooth germ and odontogenic tumors (i.e. presence or absence of α4(IV) chains) in BMs is whether the cause or effect of the epithelial-mesenchymal interaction.

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