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

Phuu Pwint Han1,2, Ryo Tamamura2, Naoki Katase2, Eiki Fujii2, Mika Okauchi2, Tan Jin3, Jing Xiao3, Chong Huat Siar4 and Hitoshi Nagatsuka2

1) Institute of Dental Medicine, Yangon, Myanmar.
2) Department of Oral Pathology and Medicine, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Japan.
3) Dental School, Dalian Medical University, China.
4) Department of Oral Pathology, Medicine and Periodontology, Faculty of Dentistry, University of Malaya, Malaysia.

(Accepted for publication, June 20, 2006)

Abstract: Type IV collagen is the principal constituent component of the basement membrane (BM). It has 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 from 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 ameloblastoma, which is locally invasive, majority of odontogenic lesions are either benign or harrmatomatous malformation (e.g., odontomas) 2-9. 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-ectomesenchymal 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,
Phuu Pwint Han et al.: Distribution of Collagen IV(α) Chains in Benign Odontogenic Tumors

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 Shigehisya 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 13 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 multipeptide 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 10mM 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% H2O2 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.

Materials and Methods

Archival formalin-fixed, paraffin embedded tissue blocks of eight ameloblastomas (five ordinary and three desmoplastic), two adenomatoïd odontogenic tumors, three ameloblastic fibromas and three odontomas were retrieved from the surgical pathology unit...
**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).

**Ameloblastoma**

All the ameloblastoma samples studied were negative to α4(IV) chains. Although no significant difference in distribution of α1(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).

**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 α 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).
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 five α(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 character of the cellular components and the morphological and functional differentiation of tumor cells compared to that of the tooth germ. However, little is known about 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 α chains composition could be observed among the BMs. Other studies which used the specific monoclonal antibody to α(IV) chains isoform were performed in mouse tooth germ and in odontogenic tumors independently. 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 α1, 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 dental 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 ameloblastoma 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. 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
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\(^\text{(3)}\). 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 hamartomatous lesion rather than 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 pseudo-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 odontogenic 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 α-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.

---

**Immunolocalization of Basement membrane Type IV collagen α1 in tooth germ and benign odontogenic tumors**

<table>
<thead>
<tr>
<th>Human tooth germ</th>
<th>Odontoma</th>
<th>AOT</th>
<th>Ameloblastoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1/2, α5/6</td>
<td></td>
<td>α4 (+)</td>
<td></td>
</tr>
<tr>
<td>α1/2, α4, α5/6</td>
<td></td>
<td>α4 (-)</td>
<td></td>
</tr>
</tbody>
</table>

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.

---

**The Schematic Diagram of the Cellular Interactions in Odontogenic Tumors Depending on the Type IV Collagen α-Chains Composition of the Basement Membrane**

- Tumors containing both odontogenic epithelium and odontogenic ectomesenchyme α4 (+) tumors
- Tumors containing odontogenic epithelium without odontogenic ectomesenchyme α4 (-) Tumors

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 parocrine 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.
Acknowledgements

This work was partially supported by grants in aid for scientific researches from the Ministry of Edition, Culture, Sports, Science and Technology (#15209060, 17406027, 17591910, 17591911).

References


