# Expression of Collagen IV α1 to α6 Chains in Basement Membranes of Odontogenic Tumors

**Hitoshi Nagatsuka[1)], Chong-Huat Siar[2)], Eiki Fujii[1)], Ryo Tamamura[1)], Naoki Katase[1)], Yasushi Morimoto[3)], and Noriyuki Nagai[1)]**

*1)Dept. of Oral Pathology and Medicine, Okayama University, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan*
*2)Dept. of Oral Pathology, Malaya Univ., Malaysia*
*3)Japan Institute for Advanced Dentistry, Japan*

Abstract: Type IV collagen, the major component of basement membrane (BM), demonstrates a stage- and position-specific distribution of its isoforms during tooth development. To determine its localization in BM of odontogenic neoplasms, immunohistochemistry using six anti-α(IV) chain-specific monoclonal antibodies was performed. Expression of α1(IV)/ α2(IV) and α5(IV)/ α6(IV) chains was stronger in the desmoplastic than in ordinary ameloblastomas. The adenomatoid odontogenic tumor distinctly expressed these chains in BM of cribriform areas and hyaline materials (which was also α4(IV)-positive. These five chains also stained BM and tumor cells of ameloblastic fibroma. Present results suggest that collagen IV composition and distribution in the basement membranes of odontogenic neoplasms plays a key role in tumor growth and progression.

Key Words: Ameloblastoma; Type IV collagen; Basement membrane.

## Introduction

Type IV collagen, the major component of mammalian BM, is a family of six distinct polypeptide chains. These are designated α1(IV) to α6(IV)) and they are encoded by six distinct genes, COL4A1 to COL4A6. These chains are assembled into triple-helical molecules composed of three α1(IV) chains that self-associate to form supramolecular networks. In human BM there are at least three molecular forms of type IV collagen which are tissue-specific in their distribution: [α1(IV)]2α2(IV) which is ubiquitous in all BM; α3(IV)α4(IV)α5(IV) which is abundant in lung alvelolar and glomerular BM; and α5(IV)/α6(IV) which is localized in the BM of mammary duct and lobule, epidermis, prostate gland and smooth muscle cells . Molecular defects in type IV collagen have been linked to Goodpasture syndrome, an autoimmune disease characterized by glomerulonephritis and pulmonary hemorrhages; Alport syndrome, a genetic disease with progressive glomerulonephritis; and diffuse esophageal leiomyomatosis characterized by benign proliferations of smooth muscle .

Odontogenesis is a complex process involving inductive and reciprocally inductive influences of odontogenic epithelium and ectomesenchyme, and the BM interposed between these two tissues functions as a regulator and modulator of these interactions. Previous studies have examined various BM constituents including collagen IV and laminin to determine their roles during tooth morphogenesis and cytodifferentation. In our earlier study on mouse molar germ development, we observed that collagen IV isoforms localized in the dental BM at various stages of tooth organogenesis showed a stage- and position-specific distribution pattern. In the light of this evidence, an immunohistochemical study utilizing α chain-specific monoclonal antibodies was carried out to determine collagen IV α chain composition and localization in the BM of ameloblastoma with the aim to clarify their roles in tumor growth and progression.

## Materials and Methods

The source of the sample studied was from the surgical pathology files of the Department of Oral Pathology and Medicine, Dentistry and Pharmaceutical Sciences, Okayama University. Archival formalin-fixed, paraffin embedded tissue blocks of 4 ameloblastomas (3 ordinary and 1 desmoplastic), 2 adenomatoid odontogenic tumors, 3 ameloblastic fibromas were retrieved and new 3-4μm sections prepared for routine staining with hematoxylin-eosin and for immunohistochemistry.

Rat monoclonal antibodies, H1l, H22, H43, M54 and M69, recognizing type IV collagen α1, α2, α3, α4, α5 and α6 chains, respectively, were raised against synthetic peptides of non-consensus amino acid sequences of the human α(IV) chains. Their specificity against the individual human α(IV) chains was confirmed by ELISA and immunoblotting, and epitopes were determined earlier by multipin-peptide scanning. All antibodies were kindly provided Dr. Ichiro NAITO and Dr. Yoshikazu SADO (Shigei Medical Research Institute, Okayama).

*Immunohistochemistry*

For immunohistochemical staining of collagen IV α1, α2, α3, α4, α5 and α6 chains, deparaffinized sections were pretreated for antigen retrieval by autoclave heating. The optimal dilutions of each primary antibody were as follows: 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 immunoreaction was performed using a Vectastatin peroxidase ABC kit (Vector Laboratories, Burlingame, CA, USA). The antigenic sites were demonstrated by reacting sections with a mixture of 0.05% 3,3-diaminobenzidine in 0.05M Tris-HCl buffer, pH 7.6, containing 0.01% H2O2 for 7 min.

## Results and Discussion

Coexpression of α1(IV) and α2(IV) chains occurred as thin

lines with limited areas of discontinuity along the BMs of neoplastic epithelial islands (follicular, Figs.1a, b) and strands (plexiform, Figs.2a, b) of ameloblastomas , but strongly stained, in linear continuous manner, the periphery of tumor nests in the desmoplastic ameloblastoma(Figs.3a, b). Collagen IV α3(IV) and 4(IV) chains were not detected in all the specimens examined. Analysis of α(IV) chain staining in the BM of ameloblastic fibroma demonstrated a uniform distribution pattern. In the tumor areas examined, α1(IV)/α2(IV), α4(IV) and α5(IV)/α6(IV) chains occurred as linear continuous patterns that compartmentalized the neoplastic epithelial islands and strands from the surrounding dental papilla-like ectomesenchymal stroma. Collagen IV α chain labeling produced distinct expression patterns in the adenomatoid odontogenic tumor. In the cribriform areas, α1(IV)/α2(IV) and α5(IV)/α6(IV) chains were strongly expressed at the interface between tumor epithelium and the hemorrhagic stroma

Although many studies have extensively investigated the cellular characteristics of odontogenic neoplasms as well as speculated on their histogenetic mechanisms, much less is known of their extracellular matrix features in particular, the BM constituents and their functions. Reports thus far focused mostly on the composition of BM in the ameloblastoma and the two BM components investigated were type IV collagen and laminin, detected immunohistochemically using either polyclonal or monoclonal antibodies directed against these macromolecules. In this study, α1 to α 6 chain-specific monoclonal antibodies were used to detect the molecular forms of type IV collagen in the BM of ameloblastoma. We demonstrated that α1(IV), α2(IV), α5(IV) and α6(IV) chains were expressed as intense stainings in the BM surrounding the neoplastic epithelial islands and strands in its distribution. We compared these expression patterns with those of oral mucosa BM and the tooth germ BM at late bell stage and found that a predominant part of the ameloblastoma BM was fundamentally similar to the BM of oral mucosa in expressing collagen IV isoforms with chain compositions [α1(IV)]2α2(IV) and α5(IV)/α6(IV). The ordinary ameloblastoma that showed limited areas of BM discontinuity, the BMs of the desmoplastic ameloblastoma appeared to exhibit an intact and uniform α(IV) chain profile. These observations correlated favorably with previous reports on collagen IV macromolecule distribution in benign odontogenic tumors and further emphasized that the presence of an intact BM is associated with a protective role. In addition, the observed intense linear staining for 1(IV)/α2(IV) and α5(IV)/α6(IV) chains in the BM of desmoplastic ameloblastoma supports Philipsen et al.'s finding which also describes a well-preserved staining pattern for collagen IV macromolecule in this tumor subset. In our study on mouse molar germ development, at the dental placode stage, the BM of the gubernaculum dentis expressed α1(IV), α2(IV), α4(IV), α5(IV) and α6(IV) chains. It suggests that ameloblastoma tumor cells with this BM expression pattern probably belonged to a more primitive phenotype i.e. resembling the gubernaculum dentis. This interpretation would in turn imply that differences in cytodifferentiation exist in the tumor cells of the ameloblastoma epithelium i.e. tumor cells associated with oral mucosa-type BM are more mature than tumor cells associated with gubernaculum dentis-type BM. Our finding indirectly supports previous studies which demonstrated that the tumor cells in the ameloblastoma do not attain the full functional differentiation status of secretory ameloblasts.

In summary, the collagen IV molecular composition in the ameloblastoma BM was clarified using α chain-specific monoclonal antibodies, and the results suggest that these BM constituents play an important role in tumor cytodifferentiation and progression.

## References


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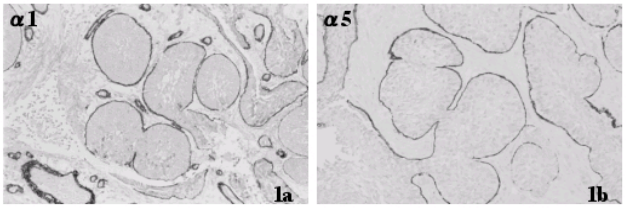


Fig. 1 a, b. Differential distribution of α (IV) chains in follicular ameloblastoma (1a, b). Immunoreactivity for collagen IVα1 (1a), α5 (1b) is observed at the periphery of tumor islands in the follicular ameloblastoma. (a, b x130)

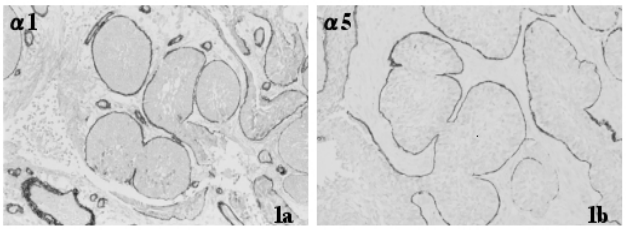


Fig.2 a, b. Differential distribution of α(IV) chains in plexiform ameloblastoma (2a, b). Immunoreactivity for collagen IV α2 (2a), α6 (2b) is observed as thin lines with limited areas of discontinuity along the BMs at the periphery of tumor islands in the plexiform ameloblastoma. (a, b x130)

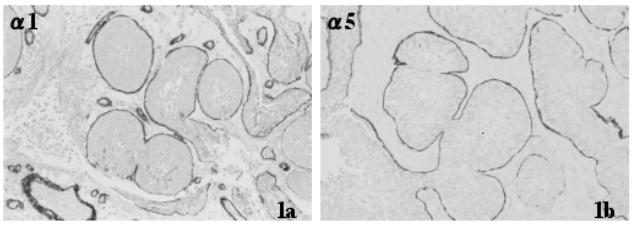


Fig.3 a, b.Differential distribution of α(IV) chains in desmoplastic ameloblastoma (3a, b). Marked immunoreactivity for collagen IV α2 (3a), α5 (3b) is observed at the periphery of tumor islands. Strongly stained, in linear continuous manner, the periphery of tumor nests in the desmoplastic ameloblastoma. (a x130, b x65)