THE EOSINOPHILIC MATERIAL IN ADENOMATOID ODONTOGENIC TUMOR ASSOCIATED WITH AMYLOID PROTEIN COMPONENT

SONG Bao-ping 宋宝萍 [1], LI Yong-mei 李咏梅 [1], Haruo Okabe 冈边治男 [2]

[1]*Department of Pathology, Shanghai Tiedao University School of Medicine, Shanghai 200070 China*
[2]*Department of Pathology, Nagasaki University School of Stomatology Nagasaki City, Japan*

Abstract

Objective: To investigate the relation between eosinophilic materials and amyloid P (AP) component in adenomatoid odontogenic tumor (AOT). Methods: The expression of amyloid proteins and basement membrane proteins, including type IV collagen, laminin and heparin sulfate proteoglycan (HSPG), in AOT were analyzed by immunohistochemical method. Results: Most eosinophilic droplets among tumor cells and some epithelial cells showed positive stain for AP component. The immunoreactions of type IV collagen and laminin were only found in blood vessels of this tumor. The tumor cells and eosinophilic materials in duct-like structures were constantly unstained for both amyloid and basement membrane proteins. Present results suggest that the nature and composition of eosinophilic droplets may differ from the eosinophilic layer in duct-like structures. This study first demonstrated that the amyloid-like deposition in AOT is associated with AP component by immunohistochemical method. It supported that AP component may be epithelial origin since the AP immunolocalization was found in tumor cells.

Key words: Adenomatoid odontogenic tumor, Amyloid protein, Basement membrane protein, Immunohistochemistry.

The adenomatoid odontogenic tumor is characterized by the formation of duct-like structures and appearance of eosinophilic materials. Eosinophilic materials show amorphous mass or droplets among the epithelial cells. In the duct-like structures, it shows a thin layer of basement membrane like structures covering the luminal surface. Recently, several ultrastructural studies indicated that eosinophilic materials are composed of various fibrils including thin collagen and masses of amyloid filaments.[1] They have been also considered as a form of enamel, dentine, cementum, dystrophic calcification and calcification of degenerate tumor cells[2] EI-Lbban suggested that the nature of eosinophilic materials be associated with degenerative blood vessels, but another study indicated that the formation of extracellular eosinophilic materials may be epithelia origin.[3] Although the AOT has been extensively studied in histology and electron microscope, the exact composition of eosinophilic materials still remains high controversy. To our knowledge, immunohistochemical studies of AOT are very rare, especially the reports about amyloid and basement membrane proteins. In this study, we investigated the expression of eosinophilic materials in AOT with immunohistochemical method in order to determine what kind of amyloid proteins present in this tumor, and whether or not basement membrane proteins participate in composition of eosinophilic materials.

Accepted for publication: October 15, 1998
Correspondence to: SONG Bao-ping; Shanghai Tiedao University School of Medicine, No. 1238, Gonghexin Road, Zhabei District, Shanghai 200070, China; Fax: (0086-21)-56901123; Phone: (0086-20)-56631160 ext. 327

MATERIALS AND METHODS

Samples

Six surgical samples of AOT were obtained from the Oral Pathology of Nagasaki University School of Stomatology. Formalin fixed, paraffin-embedded tissue were cut at a thickness of 3.5 μm sections, stained with Hematoxylin Eosin (HE) and Congo-red.

Immunohistochemistry

For immunohistochemical stain, the streptavidin peroxides conjugate (SP) method[4] was used (Histostain-SP-Kit, Zymed Laboratories Inc., South San Francisco, CA). The sections were incubated in 3%

H_2O_2 in methanol for 10 min at room temperature to block any intrinsic peroxides activity, then treated with 10% non-immune serum. The primary antibodies, employed, their sources and working dilution are listed in Table 1. After incubation of primary antibody, sections were sequentially treated with biotinylated second antibodies, fresh enzyme solution of 3-amino-9-ethylcarbazole (AEC) substrate-chromogen mixture provided in kit. All preparations were counterstained with hematoxylin. Each step was followed by three 5-min washes in PBS (10mM sodium phosphate, pH 7.5, 0.9% saline solution), except for the incubation involving normal serum. In the staining of type IV collagen, laminin and HSPG, sections were pretreated with 1% trypsin (Sigma Chemical Co.). All steps were done at room temperature.

Control

For positive controls, adenoid cystic carcinomas of salivary gland origin were used in immunostain of basement membrane proteins. In amyloid immunostains, amyloid immunoreaction was defined in amyloidosis. The negative controls without primary antibodies were run in parallel.

Table 1. Primary antibodies used in this study

Antibody	Specificity	Antibody type	Working dilution	Source
Anti-AP	Human AP component	Polyclonal	1:100	DAKO Lid.
Anti-AA	Human AA protein	Monoclonal	1:100	DAKO Lid.
Anti-LC	Free lambda chain and in intact immunoglobulin molecules	Polyclonal	1:1000	DAKO Lid.
Anti-KC	Free kappa chains and in intact immunoglobulin molecules	Polyclonal	1:100	DAKO Lid.
Anti-β 2M	Human β 2M	Polyclonal	1:100	DAKO Lid.
Anti-IV collagen	Alpha 2 derived chains of type IV collagen	Monoclonal	1:1000	Chem. Int. INC.
Anti-LAM	β I protein of intact LAM	Monoclonal	1:400	Chem. Int. INC.
Anti-HSPG	Protein core of BM BM HSPG	Monoclonal	1:200	Chem. Int. INC.

AP: amyloid P component, AA: amyloid A protein, LC: lambda light chains, KC: kappa light chains. β 2M: beta-2-microglobulin, LAM: laminin, HSPG: heparin sulfate proteoglycan, DAKO Lid: DAKO Lid., Copenhagen Denmark, Chem. Int. INC: Chemicon International, INC.

RESULTS

HE and Congo-red Staining

Six cases of AOT showed proliferative epithelial cells in solid nodules, whorls, rosettes and duct-like structures. The luminal surfaces of these duck-like structures were covered with thin layer of eosinophilic materials. The eosinophilic droplets among epithelial nests appeared in four cases (Table 2). Hyalinization and calcification were observed in some of them. Most eosinophilic materials showed positive staining for Congo-red (Figure 1), but some of them were negative for it. The tumor cells and the thin layer of eosinophilic materials covering of the luminal surface of the duct-like structures were always negative for Congo-red stain.

Immunostaining

Three of four cases stained with Congo-red showed immunostaining for AP. AP immunoreaction is observed in most of the eosinophilic droplets (Figure 2), particularly in border region of the droplets. The center region of eosinophilic droplet, with hyalinization or calcification, expressed weak immunoreactivity or unstain. Epithelial cells also showed positive staining for it. The immunoreaction was seen in cytoplasm (Figure 3). But the luminal eosinophilic materials were consistently negative. There were no positive observations in immunostaining for amyloid A (AA) component, beta-2-microglobulin, lambda and kappa light chains.

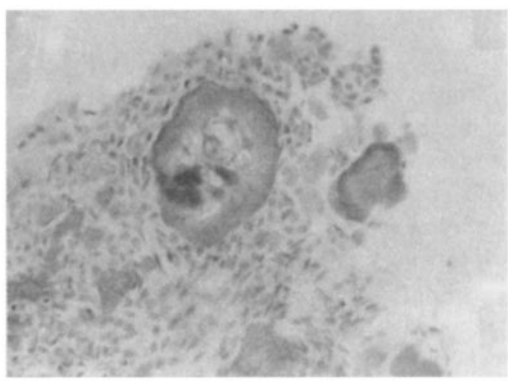

Fig. 1. The eosinophilic materials showing a Congo-red stain (×80).

Table 2. Relationship of eosinophilic droplets and amyloid stain

Case	Eosinophilic droplet	Congo-red	AP component
1	-	-	-
2	+	+	+
3	+	+	-
4	-	-	-
5	+	+	+
6	+	+	+

AP component: amyloid P component

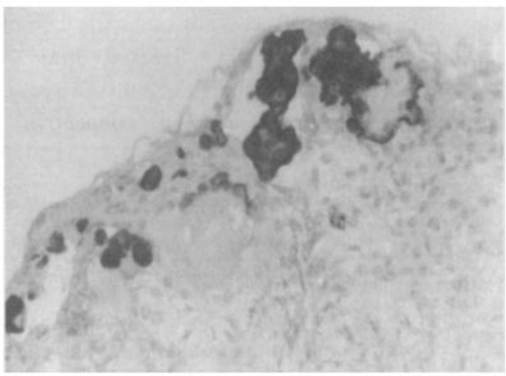

Fig. 2. The eosinophilic droplets showing positive immunoreaction for AP component (×80).

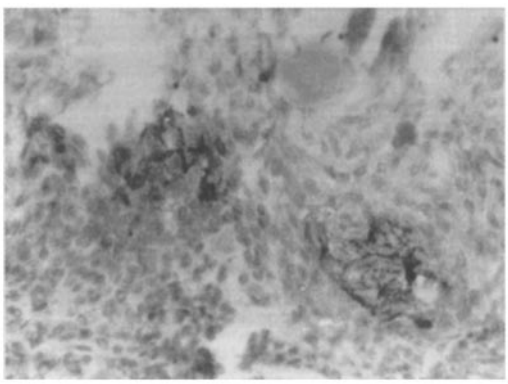

Fig. 3. The immunolocalization of AP component is noted in some tumor cells (×110).

In the immunostaining of basement membrane proteins, type IV collagen and laminin expressed positive reaction in blood vessels of tumor. We could not find immunoreaction in eosinophilic materials and tumor cells. There was no positive expression for HSPG immunostain.

DISCUSSION

Adenomatoid odontogenic tumor, a rare odontogenic neoplasm, is characterized by the columnar or cuboidal cells and frequent occurrence of amorphous deposits as well as calcifying epithelial odontogenic tumor.[5] The eosinophilic materials are also found in surface or lumen of the duct-like structures. They are considered to be an amyloid like materials, dysplastic dentine and enamel. Recent electron microscopic studies have emphasized the presence of collagen fibers, a main component of basement. The immunofluorescence study demonstrated the presence of basement membrane components including type IV collagen and laminin in the amyloid like deposits of calcifying epithelial odontogenic tumor.[6] Present study indicated that the AP component immunoreactivity and Congo-red stain are identical with appearance of eosinophilic droplets among the epithelial cells of AOT. AP is considered a minor constituent present in all forms of amyloid. It associated with may of glomerular diseases,[7] Alzheimer's disease in senile plaques[8] and several neurological disorders.[9] Recently, a subunit of AP showing antigenicity of AP, was described as a new connective tissue component.[10] In present study, AP immunoreaction suggested the amyloid deposition in eosinophilic materials of AOT. But it has been remained enigma that which kind of amyloid protein deposits in this tumor since there were no stainings for AA component, beta-2-microglobulin, lambda and kappa light chains. The immunostain of AP was also noted in some tumor cells. This founding supported the view that amyloid associated with epithelial cells. In duck-like structures, neither AP nor Congo-red stain could be noted, so the different nature of eosinophilic material among epithelia cells and in duct-like structures can be considered.

It is well known that amyloid is often stained by Congo-red, but false positive stains, including the Congo-red binding to collagen, occasional myelin sheaths and other interstitial connective tissue, have been indicated.[11] Therefore, eosinophilic materials with Congo-red stain may be composed of the other component besides the amyloid proteins.

Lambda and kappa light chains are the preproteins of amyloid protein originated in light protein. It associated with primary systemic amyloidosis and myeloma-associated amyloidosis, AA is found in secondary amyloidosis and familial mediterranean fever, and beta-2-microglobulin is found in amyloidosis associated with chronic dialysis.[12] We could not find any immunoreaction for these amyloid proteins in this study. It may be absence of these proteins in eosinophilic materials of AOT, and the other possibility is that amyloid protein antigenicity lost due to formalin fixation and paraffin embedding.

Type IV collagen, laminin and HSPG are important compositions of basement membrane. These basement membrane proteins were also found in yolk sac tumor,[13] salivary gland tumor[14] and extracellular matrix of various tumor.[15] The immuno-

fluorescence study demonstrated the presence of these proteins in calcifying epithelial odontogenic tumor.

We only found type IV collagen and laminin immunoreaction in blood vessels of AOT. Both epithelia cells and eosinophilic materials were always unstain. The ultrastructural study indicated the presence of collagen fibers in AOT. These collagen fibers may content the other type of collagen, besides the type IV collagen. In addition, amelogenin and enameling have been considered to be the important composition of eosinophilic materials since their immunolocalization were demonstrated in AOT.[16]

The present investigation first supports the eosinophilic materials of AOT associated with AP component by immunohistochemical method. The AP component may be epithelial origin since its immunolocalization was found in tumor cells.

REFERENCES

[1] EI-Labban NG. The nature of the eosinophilic and laminated masses in the adenomatoid odontogenic tumor: a histochemical and ultrastructural study. J Oral Pathol Med 1992; 21:75.

[2] Shear M. The histogenesis of the tumor of enamel organ epithelium. Br Dent J 1962; 112:494.

[3] Smith RRL, Olson JL, Hutchins GM, et al. Adenomatoid odontogenic tumor: ultrastructural demonstration of two cell types and amyloid. Cancer 1979; 43:505.

[4] Shi ZR, Itzkowitz SH, Kin YS. A comparison of three immunoperoxidase techniques for antigen detection in colorectal carcinoma tissue. J Histochem Cytochem 1988; 36:317.

[5] Moro I, Okamura N, Okuda S, et al. The eosinophilic and amyloid-like materials in adenomatoid odontogenic tumor. J Oral Pathol 1982; 11:138.

[6] Sauk JJ, Cocking-Johnson D, Warings M. Identification of basement membrane components and intermediate filaments in calcifying epithelial odontogenic tumors. J Oral Pathol 1985; 14:133.

[7] Yang GCH, Nieto R, Stachura I, et al. Ultrastructural immunohistochemical localization of polyclonal IgG, C3, and amyloid P component on the Congo-red negative amyloid-like fibrils of fibrillary glomerulopathy. Am J Pathol 1992; 141:409.

[8] Kalaria RN, Grahovac I. Serum amyloid P immunoreactivity in hippocampal tangles, plaques and vessels: implications for leakage across the blood-brain barrier in alzheimers diseased. Brain Res 1990; 516:349.

[9] Akiyama H, Yamada T, Kawamata T, et al. Association of amyloid P component with complement proteins in meurogically diseased brain tissue. Brain Res 1991; 548:349.

[10] Inooue S. Pentosome-a new connective tissue component-is a subunit of amyloid P. Cell Tissue Res 1991; 263:431.

[11] Carson FL, Kingsley WB. Nonamyloid green birefringence following Congo-red staining. Arch Pathol Lab Med 1980; 104:333.

[12] Shirahama S, Skinner M, Cohen AS, et al. Histochemical and immunohistochemical characterization of amyloid associated with chronic hemodialysis as beta-2-microglobulin. Lab Invest 1985; 53:705.

[13] Barsky SH, Layfield L, Varki N, et al. Two human tumors with high basement membrane producing potential. Cancer 1988; 61:1798.

[14] Skalova A, Leivo I. Basement membrane proteins in salivary gland tumors. Virchows Archiv A Pathol Anat 1992; 420:425.

[15] Liotta LA, Rao CN, Barsky SH. Tumor invasion and the extracellular matrix. Lab Invest 1983; 49:636.

[16] Saku T, Okabe H, Shimokawa H. Immunohistochemical demonstration of enamel proteins in odontogenic tumors. J Oral Pathol Med 1992; 21:113.