Midkine expression correlating with growth activity and tooth morphogenesis in odontogenic tumors

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Abstract

Midkine (MK; a low molecular weight heparin-binding growth factor) is a multifunctional cytokine. MK plays a role in morphogenesis of many organs including teeth through epithelial-mesenchymal interactions. We immunohistochemically examined MK expression in various human odontogenic tumors. There was no difference in positive rate and intensity of MK between benign odontogenic tumors and their malignant counterparts. Ameloblastoma showed MK localization in the peripheral columnar cells in budding processes from the parenchyma, which frequently expressed proliferating cell nuclear antigen (PCNA). MK was also preferentially expressed in keratinized cells in acanthomatous ameloblastoma and keratocystic odontogenic tumor. In odontogenic mixed tumors except for odontoma, intense immunoreactivity to MK was found in epithelial follicles, the surrounding odontogenic ectomesenchymal tissue, and the basement membrane between them. Intensity in the odontogenic ectomesenchyme decreased in relation to distance from the epithelial follicles. No expression was found in tumor cells associated with production of dental hard tissues in odontogenic mixed tumors including odontoma. These findings suggested that MK is involved in the reciprocal interaction between odontogenic epithelium and odontogenic ectomesenchymal tissue in areas without dental hard tissue formation in odontogenic mixed tumors. Co-expression of MK and PCNA was also observed in epithelial follicles and highly cellular nodules in the

ectomesenchyme of odontogenic mixed tumors. MK is considered to mediate growth activity of odontogenic tumors and cell differentiation of odontogenic mixed tumors through molecular mechanisms similar to those involved in morphogenesis of the tooth.

Abbreviations: MK, midkine; PCNA, proliferating cell nuclear antigen

1. Introduction

Midkine (MK) is a low molecular weight heparin-binding growth factor. This cytokine is highly expressed in the mid-gestational period during embryogenesis. MK participates in cell growth, survival, migration, neurogenesis, and carcinogenesis. MK is involved also in morphogenesis of various organs through epithelial-mesenchymal interactions. [1-6]

In the morphogenesis of the tooth, enamel and dentin matrices are produced by ameloblasts and odontoblasts, respectively. Ameloblasts are derived from odontogenic epithelial elements, and odontoblasts differentiate from odontogenic ectomesenchymal tissue in the tooth germ. These epithelial and mesenchymal differentiations do not independently occur, but are induced through the intermediary interaction between odontogenic epithelial and ectomesenchymal components. [7,8] Therefore, tooth germ is regarded as one of the good morphogenetic models for epithelial-mesenchymal interactions. Mitsiadis et al. observed the regulation of MK gene and protein expression by epithelial-mesenchymal interaction in the tooth germ, and suggested an important role for MK in the molecular cascade that controls tooth development. [9]

The MK expression in odontogenic tumors has not been investigated. Odontogenic tumors are histologically divided by WHO into several categories, depending on their constituent components, as follows: [10]

Benign

1) Tumors comprising odontogenic epithelium with mature, fibrous stroma without odontogenic ectomesenchyme

2) Tumors comprising odontogenic epithelium with odontogenic ectomesenchyme, with or without dental hard tissue formation

3) Tumors comprising mesenchyme and/or odontogenic ectomesenchyme with or without odontogenic epithelium

Malignant

1) Odontogenic carcinomas

2) Odontogenic sarcomas

Ameloblastoma, ameloblastic carcinoma, keratocystic odontogenic tumor, and adenomatoid odontogenic tumor are composed of odontogenic epithelium with mature, fibrous stroma without odontogenic ectomesenchyme. Ameloblastic fibroma and ameloblastic fibrosarcoma are tumors characterized by odontogenic epithelium with odontogenic ectomesenchyme, without dental hard tissue formation. Odontoma, ameloblastic fibrodentinoma, and ameloblastic fibro-odontoma are categorized as tumors comprised of odontogenic epithelium with odontogenic ectomesenchyme, with dental hard tissue formation. Almost all tumors possessing dental hard tissue formation contain neoplastic ameloblasts and/or odontoblasts associated with production of enamel and dentin matrices, respectively.

To investigate whether MK participates in the construction of characteristic histological structures in these tumors, we looked at their MK immunoexpression.

2. Materials and methods

We investigated 141 cases of human odontogenic tumors including ameloblastoma

(55), ameloblastic carcinoma (3), keratocystic odontogenic tumor (28), adenomatoid odontogenic tumor (4), odontoma (compound type (24) and complex type (15)), ameloblastic fibroma (3), ameloblastic fibrodentinoma, ameloblastic fibro-odontoma (7), and ameloblastic fibrosarcoma (2). Specimens were retrieved from the histopathological files of the Division of Oral Pathology and Bone Metabolism, Unit of Basic Medical Sciences, Course of Medical and Dental Sciences, Nagasaki University Graduate School of Biomedical Sciences. The specimens were fixed in 10% neutral buffered formalin fixative, decalcified with formic acid where necessary, embedded in paraffin, cut into 3-μm-thick sections, and stained with hematoxylin and eosin (HE). Table 1 summarizes the clinical data of all the patients.

For the investigation of MK expression, we employed a mouse anti-human MK monoclonal antibody (TFB Inc., Tokyo, Japan) as the primary antibody. To evaluate the relationship with cell growth, a mouse anti-human proliferating cell nuclear antigen (PCNA) monoclonal antibody (DakoCytomation Co., Ltd., Kyoto, Japan) was applied after the retrieval of PCNA in 0.01 M citrate buffer (pH: 6.0) by heating (121°C, 10 min) in an autoclave. Immunostaining was carried out using the EnVision + system (DakoCytomation). The chromogen 3,3'-diaminobenzidine (Sigma-Aldrich Japan, Tokyo) was used to reveal the sites of peroxidase activity. After counterstaining the sections with hematoxylin, they were dehydrated and mounted in a synthetic mounting medium.

3. Results

A brief summary of the results is shown in Table 2.

3.1. Ameloblastoma

MK localization was found in 30 of 55 ameloblastomas (54.5%). The columnar epithelial cells at the periphery of the parenchyma, especially in cells projecting from epithelial nests, showed immunoreaction with MK in their cytoplasm. However, not all peripheral cells were uniformly positive; patchy positivity was observed in the periphery of the nests. Faint or absent expression were noted in the fibroblasts of the stroma (Fig. 1a). MK was expressed also in squamous metaplastic areas or the acanthomatous patterns of ameloblastoma, which were composed of epithelial cells with eosinophilic abundant cytoplasm suggesting keratinization (Fig. 1b). PCNA was frequently expressed in the nuclei of the peripheral columnar cells rather than the central cells. Particularly columnar cells in projections budding from the nests exhibited intense immunostaining for PCNA (Fig. 1c).

3.2. Ameloblastic carcinoma

Two of 3 ameloblastic carcinomas were positive for MK (66.7%), with localization in practically the entire peripheral columnar epithelium of tumor parenchyma. Stroma showed no MK expression. There was no noticeable difference in positive rate and staining intensity between benign ameloblastomas and ameloblastic carcinomas.

3.3. Keratocystic odontogenic tumor

MK was expressed in 11 of 28 keratocystic odontogenic tumors (39.3%). We found intense reactivity in the neoplastic epithelium and exfoliated keratin (Fig. 2). Invaginated epithelium into the fibrous stroma from keratinized squamous epithelium lining the cyst wall, and epithelium of daughter cysts, showed immunolocalization.

3.4. Adenomatoid odontogenic tumor

No MK expression was seen in the parenchyma and stroma in all cases.

3.5. Odontoma

We observed MK expression only in 1 of 24 compound type odontomas (4.2%) and 2 of 15 complex type odontomas (13.3%). There was no expression in dental hard tissues, reduced ameloblasts, differentiated pulp cells, and odontoblasts (Fig. 3), whereas small nodules of odontogenic ectomesenchymal tissue and round islands of odontogenic epithelium in the tumor had MK expression. The latter cellular components were not associated with production of dental hard tissues.

3.6. Ameloblastic fibroma

Immunoreactivity was stronger in all ameloblastic fibromas than ameloblastomas. MK was expressed in follicular epithelial elements like the enamel organ of tooth germ and surrounding odontogenic ectomesenchymal tissue. The positive tumor cells exhibited cytoplasmic and nuclear reaction. The intensity of expression in the ectomesenchyme was weaker with increasing distance from the epithelial follicles (Fig. 4a). Basement membrane circumscribing epithelial follicles and some highly cellular areas of odontogenic ectomesenchyme were strongly MK positive (Fig. 4b). PCNA was expressed both in epithelial and ectomesenchymal tissues. Especially, peripheral columnar cells were strongly positive, whereas the ectomesenchymal cells adjacent to the epithelial follicles infrequently showed reactivity to PCNA. In the ectomesenchymal area, highly cellular nodules demonstrated intense PCNA expression with distribution similar to that of MK (Fig. 4c).

3.7. Ameloblastic fibrodentinoma and fibro-odontoma

All ameloblastic fibrodentinomas and fibro-odontomas were positive for MK, and its distribution in areas without dental hard tissue formation was similar to that of ameloblastic fibroma. Participants in hard tissue production, including epithelial islands and ectomesenchymal cells adhering to or near the dental hard tissue, showed very faint or no expression of MK. Basement membrane around the epithelial elements in this hard tissue-producing area exhibited no expression (Fig. 5).

3.8. Ameloblastic fibrosarcoma

All ameloblastic fibrosarcomas showed strong MK expression in both the epithelial follicles and surrounding ectomesenchymal components. Although the ectomesenchymal tissues exhibited diffuse MK expression, no difference in intensity of MK expression was noted between ameloblastic fibroma and ameloblastic fibrosarcoma.

4. Discussion

MK is involved in carcinogenesis and cancer-related angiogenic, fibrinolytic, chemotactic, mitogenic, anti-apoptotic, and transforming activities. [1,2,4] Therefore, various carcinomas express MK, and serum MK level increases in some carcinomas including hepatocellular carcinoma and gastrointestinal carcinomas. [11-13] In the present study, there was no difference in positive rate or intensity of MK expression between benign odontogenic tumors and their malignant counterparts (Table 2). These data suggest that MK cannot be used as a marker to distinguish malignant from nonmalignant odontogenic tumors.

Sandra et al. found immunohistochemical expression of MK in 70% of

ameloblastoma cases and high MK labeling index in the outer cells of the parenchyma. [14] They concluded that MK played an important role in the growth of ameloblastoma, after observing that the level of MK expression reflected the growth rate of ameloblastoma. We also preferentially detected MK expression in columnar epithelium projecting from the parenchyma of ameloblastomas. WHO defined solid/multicystic type ameloblastoma (conventional ameloblastoma) as a slowly growing, locally invasive, epithelial odontogenic tumor of the jaws with a high rate of recurrence if not removed adequately, but with virtually no tendency to metastasize. [10] These MK-positive cells were virtually all PCNA-positive and suggested that MK was associated with local aggressiveness and/or growth activity of odontogenic tumors. The absence of MK expression in adenomatoid odontogenic tumors, which are usually nonaggressive, supports this hypothesis.

Interestingly, relatively strong expression of MK was detected in squamous metaplastic areas of the acanthomatous ameloblastoma. Keratinized odontogenic epithelium was MK positive in 39.3% of keratocystic odontogenic tumors. MK may participate in keratinization of epithelial cells, because it is expressed more intensely in well-differentiated than poorly differentiated squamous cell carcinoma in esophageal and vulvar regions. [15,16] In addition to the epithelium with exfoliated keratin, MK expression was observed in invaginated epithelium from cyst wall and epithelium of daughter cysts. These findings suggest that the expression profile of MK correlates with local aggressiveness and/or growth activity of keratocystic odontogenic tumors.

MK expression also correlates closely with morphogenesis of various organs regulated through epithelial-mesenchymal interactions. [1-6] Physiological morphogenesis

and cell differentiation in tooth development are regulated by a series of epithelial-mesenchymal interactions. [7,8,17] Many transcription and signaling factors are associated with reciprocal interactions involved in tooth development. [18-27] Various odontogenic tumors histologically mimic the odontogenic tissues that participate in tooth development. Thus some factors involved in tooth development have been immunohistochemically revealed in odontogenic tumors. [28-34] Mitsiadis et al. [9] investigated MK expression in tooth development of mice and found that the distribution of MK expression depended on the developmental stage of the tooth germ. In the late bell stage without dental hard tissue formation, intense MK localization was found in the inner enamel epithelium, whole dental papilla, and the basement membrane between them. In contrast, in the late bell stage with dental hard tissue formation, MK expression was absent from all dental cells in the crown region. Therefore, inner enamel epithelium and differentiating ectomesenchymal cells showed MK localization, whereas MK localization in ameloblasts, odontoblasts, and pulp cells diminished after their terminal differentiation.

The distribution of MK in ameloblastic fibroma and ameloblastic fibrosarcoma closely resembled that in the bell stage without dental hard tissue formation, and MK localization in the basement membrane indicated signaling of reciprocal interaction between epithelial follicles and ectomesenchymal tissue. [9] Odontoma is regarded as a hamartoma and also mixed tumor histologically composed predominantly of dental hard tissues, i.e., dentin, enamel, and cementum. It contains also a small amount of odontogenic epithelium and ectomesenchyme. Its cellular elements include reduced ameloblasts, odontoblasts, and pulp cells after terminal differentiation. [10] In our study, the tumor cells

adjacent to the dental hard tissues in odontoma showed no MK expression. In ameloblastic fibrodentinoma and fibro-odontoma, no MK localization occurred in the epithelial or mesenchymal cells adhering to the hard tissue, which were thought to take part in formation of the hard tissues. It is indicated that MK participates in the process of differentiation, and its expression decreases upon completion of differentiation and acquisition of function in odontogenic tumors, corresponding to the development of the mouse tooth. [9] Moreover, PCNA was frequently expressed in epithelial follicles and ectomesenchymal cells present in the high cellularity area of the odontogenic mixed tumor, and similar in distribution to that of MK expression. Co-expression of MK and PCNA suggested that MK is also involved in cell growth of odontogenic mixed tumors other than odontoma.

In conclusion, no difference in MK-positive rate and intensity was revealed between benign odontogenic tumors and their malignant counterparts, but our results suggest that the level of expression was correlated with local growth activity of odontogenic tumors. MK was frequently expressed in odontogenic mixed tumors except for odontoma, which contain tumor cells that have not completely differentiated. It is suggested that MK mediates epithelial-mesenchymal interactions in odontogenic mixed tumors as well as in physiological odontogenesis.

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Table 1. Odontogenic tumors included in this study

Histology	No. of cases	Sex		Average age at first visit
		Male	Female	
Ameloblastoma	55	33	22	42.8
Ameloblastic carcinoma	3	2	1	66.7
Keratocystic odontogenic tumor	28	15	13	34.0
Adenomatoid odontogenic tumor	4	1	3	23.5
Odontoma	39	16	23	24.0
Compound	(24	8	16	20.9)
Complex	(15	8	7	29.0)
Ameloblastic fibroma	3	2	1	19.0
Ameloblastic fibrodentinoma and fibro-odontoma	7	4	3	13.0
Ameloblastic fibrosarcoma	2	1	1	26.5
total	141	74	67	

Table 2. Expression of MK in odontogenic tumors

Histology (no. of cases)	No. of positive cases	Localization
Ameloblastoma (55)	30 (54.5%)	peripheral columnar cells squamous metaplasia
Ameloblastic carcinoma (3)	2 (66.7%)	peripheral columnar cells squamous metaplasia
Keratocystic odontogenic tumor (28)	11 (39.3%)	squamous epithelium
Adenomatoid odontogenic tumor (4)	0 (0%)	
Odontoma (39)	3 (7.7%)	
Compound (24)	1 (4.2%)	islands of odontogenic epithelium*
Complex (15)	2 (13.3%)	islands of odontogenic epithelium and ectomesenchyme*
Ameloblastic fibroma (3)	3 (100%)	epithelial elements and their surrounding odontogenic ectomesenchyme
Ameloblastic fibrodentinoma and fibro-odontoma (7)	7 (100%)	epithelial elements and their surrounding odontogenic ectomesenchyme*
Ameloblastic fibrosarcoma (2)	2 (100%)	epithelial elements and their surrounding odontogenic ectomesenchyme

* These tissues are not associated with dental hard tissue formation.

Figure legends

Figure 1. Ameloblastoma expressing MK (a, b) and PCNA (c). (a) Partial peripheral columnar epithelium shows cytoplasmic expression of MK. Stromal fibroblasts exhibit weak immunoreaction. (b) Large keratinocyte-like cells in squamous metaplastic areas also demonstrate intense expression. (c) The cells in the projections budding from epithelial nests are strongly positive for PCNA.

Figure 2. Keratocystic odontogenic tumor. MK expression is found in the neoplastic epithelium and exfoliated keratin materials.

Figure 3. Mature dentin and pulp in compound odontoma immunostained with MK. No expression is observed in pulp cells, odontoblasts, dentinal fibers, and dentin.

Figure 4. Ameloblastic fibroma expressing MK (a, b) and PCNA (c). (a) Intense immunoreaction with MK is observed in epithelial follicles (E) and surrounding odontogenic ectomesenchymal tissue. Intensity of expression is weak away from the epithelium. (b) Epithelial components and accumulation of odontogenic ectomesenchymal cells show strong MK expression. Basement membrane around the epithelial follicles is also positive. (c) Epithelial follicles and the highly cellular area in ectomesenchymal tissue demonstrate strong immunoreaction to PCNA.

Figure 5. Immature dentin (D) in ameloblastic fibrodentinoma. MK is not localized in both the odontogenic epithelium (arrows) and odontogenic ectomesenchymal cells (arrow heads) near the dentin.

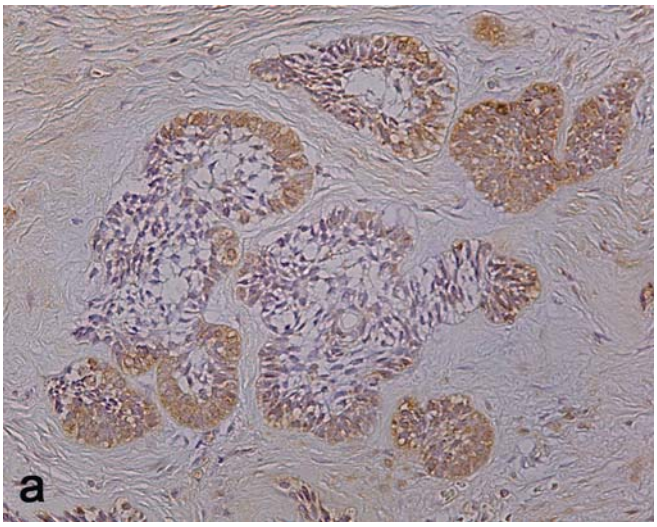

Fig. 1a

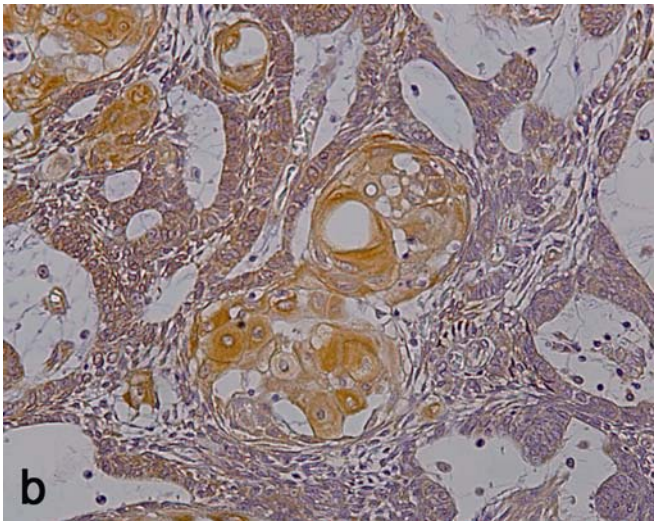

Fig. 1b

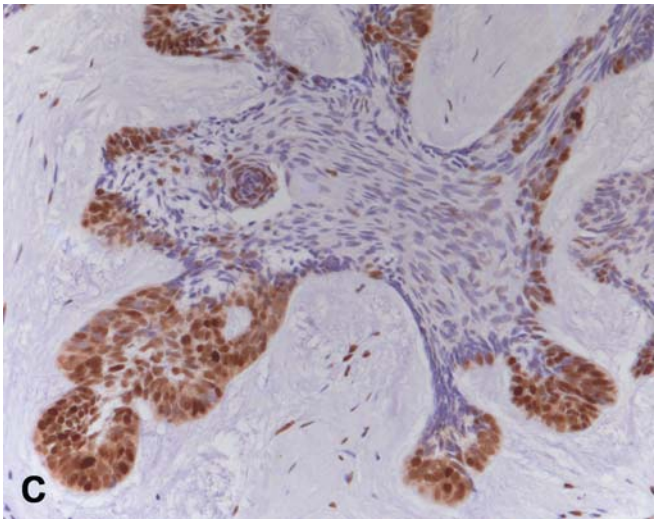

Fig. 1c

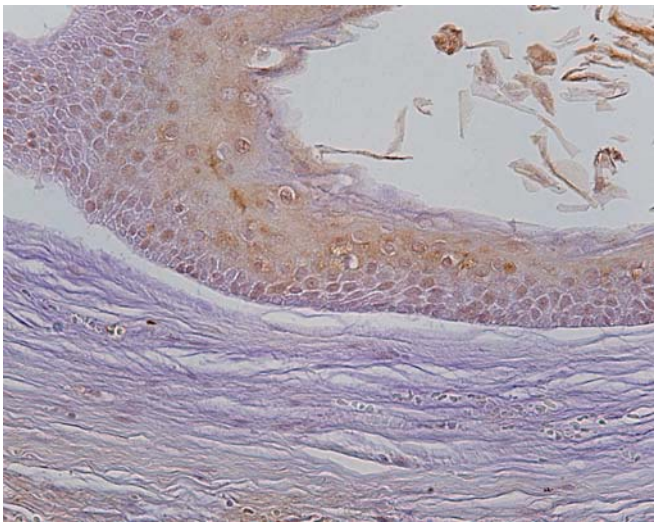

Fig. 2

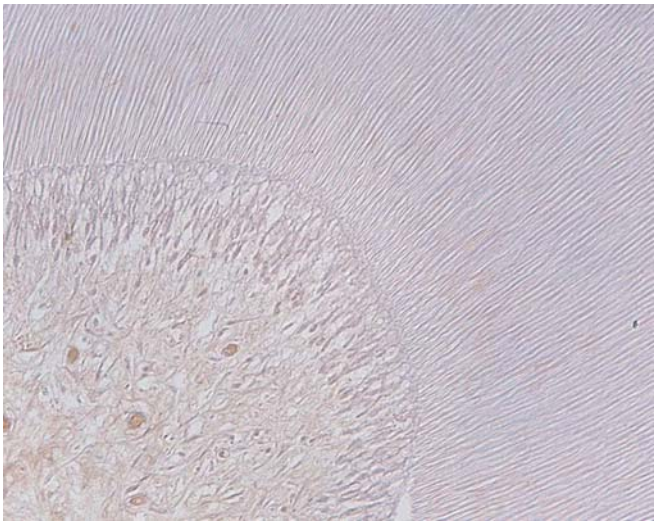

Fig. 3

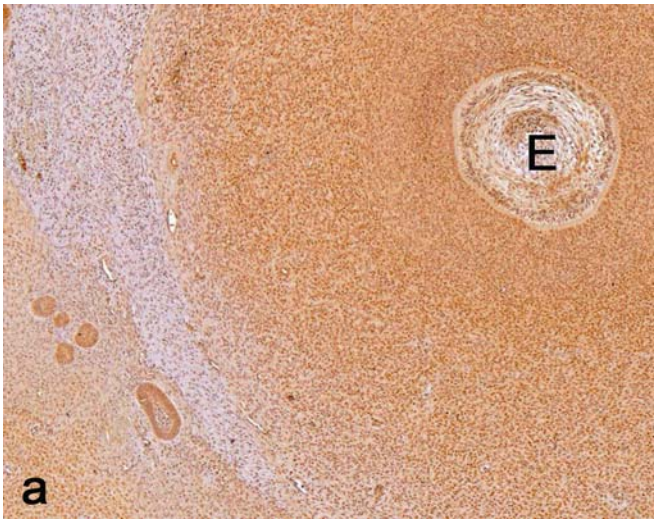

Fig. 4a

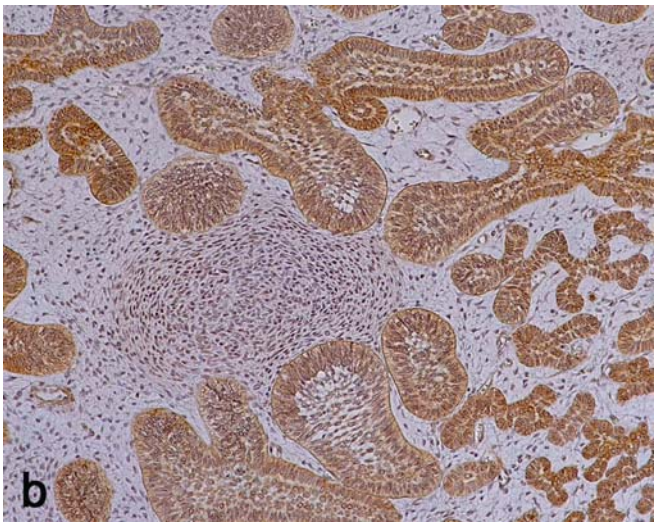

Fig. 4b

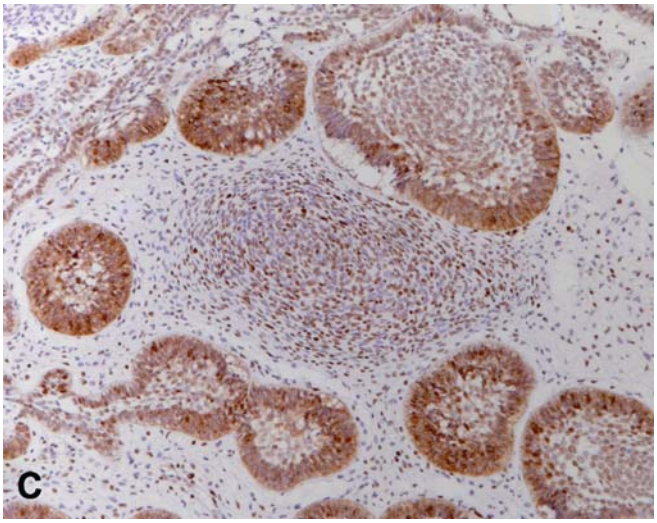

Fig. 4c

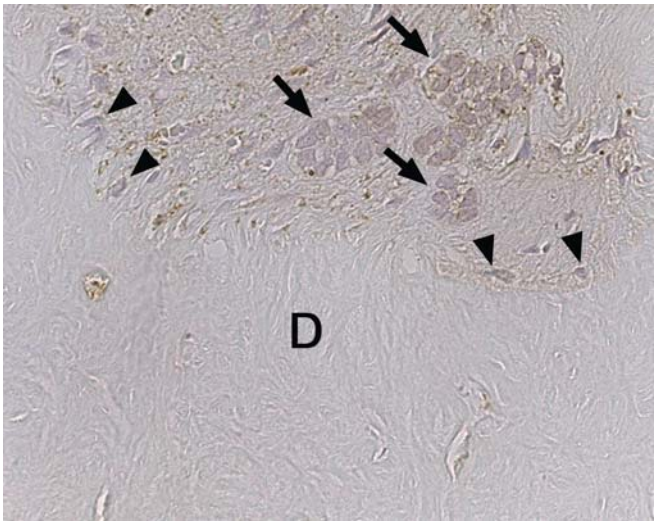

Fig. 5