ORIGINAL ARTICLE

Nestin expression in odontoblasts and odontogenic ectomesenchymal tissue of odontogenic tumours

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Background: Nestin, one of the intermediate filaments constituting the cytoskeleton, is a marker of neural stem cells or progenitor cells. Its expression is also related to tooth development and repair of dentine.

Aims: The aim of this study was to investigate nestin expression in various odontogenic tumours and evaluate its usefulness for histopathological diagnosis.

Methods: We studied formalin fixed, paraffin embedded specimens from 129 cases of odontogenic tumours and 9 of mandibular intraosseous myxoma. After characterisation of odontogenic ectomesenchymal tissues in these tumours using antibodies to vimentin, desmin, neurofilament, and glial fibrillary acidic protein, we immunohistochemically examined nestin expression.

Results: No differentiation towards muscle and nervous tissues was found in the odontogenic ectomesenchymal tissues. Although almost all the ameloblastomas and malignant ameloblastomas were negative for nestin, odontogenic ectomesenchyme in the odontogenic mixed tumours demonstrated nestin immunolocalisation, particularly in the region adjacent to the odontogenic epithelium. Odontoblasts and their processes, pulp cells near the positive odontoblasts, and flat cells adhering to the dentine showed immunoreaction with nestin in the odontomas and odontoma-like component in the ameloblastic fibro-odontomas. Neoplastic cells in almost half cases of jaw myxoma and one case of odontogenic fibroma expressed nestin.

Conclusions: The distribution of nestin in the odontogenic mixed tumours suggests that nestin expression in the odontogenic ectomesenchyme is upregulated by stimulation from odontogenic epithelium. In addition, nestin may also be involved in the differentiation from pulp cells to odontoblasts in odontogenic tumours. Therefore, nestin is a useful marker for the odontogenic ectomesenchyme and odontoblasts in odontogenic tumours.

Nestin, one of the intermediate filaments constituting the cytoskeleton, is a marker of neural stem cells or progenitor cells. Its expression is also related to tooth development and repair of dentine.

Nestin is one of the intermediate filaments constituting the cytoskeleton and is known as a marker of neural stem cells or progenitor cells.¹ This protein appears at an early developmental stage of both the central nervous system and muscles. Although the function of nestin in cell differentiation is not clearly understood, nestin is replaced with glial fibrillary acidic protein (GFAP) and desmin, accompanied by developmental processing of nervous and muscular tissues, respectively.^{1–3} Nestin expression has been reported in other tissues and pathological conditions, such as neural crest,¹ heart,⁴ testis,⁵ reactive astrocytes after brain injury,^{6–9} and central and peripheral nervous system tumours.^{10–12}

With respect to tooth development and dental pathological conditions, odontoblasts producing dentine in the human and rodent tooth germ express nestin.^{13–14} Dentinal fibres and pulp cells adjacent to the odontoblasts at the tooth cusp regions also indicate localisation of nestin. Human nestin expression continues during tooth development and disappears when development is complete.¹³ In the mature tooth with caries or after cavity formation, nestin is re-upregulated in odontoblasts and their processes when they are stimulated by caries or when they form the secondary dentine after injury, respectively.^{13–16} Thus, nestin is considered to be related to the differentiation toward odontoblasts and to the acquisition of the function to produce dentine.

In this study, we immunohistochemically investigated nestin expression in varied odontogenic tumours. Odontogenic neoplasms are histologically divided into several categories depending on their constituting components by

WHO as detailed in table 1.¹⁷ The neoplasms indicated within parentheses were the objective materials of our study, although there is no conclusive evidence that the jaw myxomas examined in our study are derived from odontogenic organs. We found a characteristic nestin distribution in all these tumours and discussed the implication of its expression in the odontogenic tumours.

MATERIALS AND METHODS

We investigated 129 cases of human odontogenic tumours: ameloblastoma (44), malignant ameloblastoma (3), odontoma (compound type 40; complex type 22), ameloblastic fibroma (2), ameloblastic fibro-odontoma and fibrodentinoma (7), ameloblastic fibrosarcoma (2), adenomatoid odontogenic tumour (AOT) (6), and odontogenic fibroma (3). In addition, nine cases of intraosseous myxoma in the mandible were also examined. These materials were retrieved from the histopathological files of Division of Oral Pathology and Bone Metabolism, Department of Developmental and Reconstructive Medicine, 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 haematoxylin and eosin. Table 2 summarises the clinical data of all the patients.

To confirm that the odontogenic ectomesenchymal tissues in these tumours contain no nervous and muscular tissues,

Abbreviations: AOT, adenomatoid odontogenic tumour; GFAP, glial fibrillary acidic protein

Table 1 Categories of tumour

Benign
Tumours comprising odontogenic epithelium without odontogenic ectomesenchyme (for example, ameloblastoma)
Tumours comprising odontogenic epithelium with odontogenic ectomesenchyme, with or without dental hard tissue formation (for example, ameloblastic fibroma, ameloblastic fibro-odontoma, ameloblastic fibrodentinoma, odontoma and adenomatoid odontogenic tumour)
Tumours comprising odontogenic ectomesenchyme with or without odontogenic epithelium (for example, odontogenic fibroma and myxoma)
Malignant
Odontogenic carcinomas (for example, malignant ameloblastoma)
Odontogenic sarcomas (for example, ameloblastic fibrosarcoma)
Odontogenic carcinosarcoma

Table 2 Examined odontogenic tumours and jaw myxoma

Histology	No. of cases	Sex		Average age at first visit
		Male	Female	
Ameloblastoma	44	26	18	35.4
Malignant ameloblastoma	3	2	1	53.0
Odontoma	62	29	33	23.2
Compound type	40	17	23	21.1
Complex type	22	12	10	26.7
Ameloblastic fibroma	2	1	1	21.0
AFO and AFD	7	5	2	13.3
Ameloblastic fibrosarcoma	2	1	1	26.5
AOT	6	2	4	19.8
Odontogenic fibroma	3	1	2	36.7
Myxoma	9	5	4	41.7
Total	138	72	66	

AFO and AFD, ameloblastic fibro-odontoma and fibrodentinoma; AOT, adenomatoid odontogenic tumour.

immunostaining for vimentin, desmin, neurofilament, and GFAP were performed, because muscular and neural cells frequently react with antibodies to nestin. All monoclonal antibodies against these proteins were purchased from DakoCytomation Co. Ltd, Kyoto, Japan. The immunohistochemical assay was performed using the EnVision+ system (DakoCytomation) with goat anti-mouse immunoglobulin conjugated to peroxidase labelled dextran polymer.

For the investigation of nestin expression, we used rabbit anti-human nestin polyclonal antibody (Chemicon International Inc., CA, USA) as a primary antibody, and the EnVision+ system (DakoCytomation) for polyclonal antibody.

The chromogen 3,3'-diaminobenzidine (Sigma-Aldrich Japan, Tokyo, Japan) was used to reveal the sites of peroxidase activity. After the sections were counterstained with haematoxylin, they were dehydrated and mounted in a synthetic medium.

RESULTS

Characterisation of odontogenic ectomesenchymal tissues

Odontogenic ectomesenchyme and pulp tissue included in the odontogenic mixed tumours, and the odontomas were positive for vimentin and negative for desmin, neurofilament, and GFAP (supplemental fig 1; <http://www.jclinpath.com/supplemental>). We found that intrinsic muscle and nervous tissues showed desmin expression, and neurofilament and GFAP expression, respectively (supplemental fig 2; <http://www.jclinpath.com/supplemental>).

Immunohistochemical assay for nestin

A brief summary of the results is shown in table 3.

Ameloblastoma

The parenchyma of almost all the cases (43 of 44) of ameloblastoma displayed no nestin immunoreactivity (fig 1). However, one case showed the expression in the cytoplasm of the focal peripheral columnar epithelium bordering the fibrous stroma.

Figure 1 Parenchyma and stroma cells of ameloblastoma showed no nestin immunoreactivity.

Table 3 Expression of nestin in odontogenic tumours and jaw myxoma

Histology (no. of cases)	No. of positive cases	Localisation in parenchyma
Ameloblastoma (44)	1 (2%)	Peripheral columnar cells
Malignant ameloblastoma (3)	0 (0)	No localisation
Odontoma (62)	49 (79)	
Compound type (40)	33 (82)	Odontoblasts, dentinal fibres, pulp cells associated with odontoblasts
Complex type (22)	16 (73)	Cells adhering to dentine, their processes in dentinal tubules
Ameloblastic fibroma (2)	2 (100)	Ectomesenchyme > epithelium
AFO (7) and AFD	6 (86)	Ectomesenchyme > epithelium
Ameloblastic fibrosarcoma (2)	2 (100)	Ectomesenchyme
AOT (6)	5 (83)	Small nodular foci and rosette patterns
Odontogenic fibroma (3)	1 (33)	Ectomesenchyme
Myxoma (9)	4 (44)	Tumour cells homogeneously in positive cases

AFO and AFD, ameloblastic fibro-odontoma and fibrodentinoma; AOT, adenomatoid odontogenic tumour.

Figure 2 Odontoma showed nestin expression in the odontoblasts and their processes in the dental tubules. Pulp cells adjacent to the odontoblasts gave a positive reaction (arrows).

Figure 3 Ameloblastic fibroma, consisting of odontogenic epithelial follicle (Ep) and ectomesenchymal tissue (Ec). Nestin was expressed in the odontogenic ectomesenchymal cells, particularly around the epithelial component.

Malignant ameloblastoma

No localisation of nestin was observed in the parenchyma of all three cases of malignant ameloblastoma.

Odontoma

Nestin immunoreactivity was found in the columnar odontoblasts adjacent to the dentine matrix, particularly in the compound-type odontomas. The positive odontoblasts were arranged in a palisading pattern on the predentine or dentine. Dentinal fibres through the dental tubules extending from the odontoblasts also showed immunoreactivity, and pulp cells adjacent to the odontoblasts were positive for nestin in some cases (fig 2). In addition, in complex-type odontomas, sparse flat cells adhering to the dentine and their processes in the irregular dental tubules were positive. Of 40 compound odontomas, 33 cases were positive, as were 16 of 22 complex odontomas.

Ameloblastic fibroma

Odontogenic ectomesenchymal tissues in the ameloblastic fibromas demonstrated immunoreactivity for nestin,

Figure 4 Dentinoid matrix included in the ameloblastic fibro-dentinoma. The odontogenic epithelial component (Ep) is adjacent to the dentinoid. Intense reactivity with nestin was found in the plump cells adhering to the dentinoid (arrows) and dentinal fibres (arrow heads).

although they did not contain the differentiated odontoblasts. Foci of aggregation of positive cells were noted in the ectomesenchymal element. As an intriguing finding, the ectomesenchymal cells around the neoplastic follicular odontogenic epithelium showed intense reaction with nestin, and the intensity weakened with greater distance from the odontogenic epithelium (fig 3). Some follicular epithelial components showed a cytoplasmic reaction in the peripheral columnar and inner stellate cells.

Ameloblastic fibro-odontoma and fibro-dentinoma

The localisation of nestin in the ameloblastic fibro-odontomas and ameloblastic fibro-dentinomas mimicked that in the ameloblastic fibromas and odontomas. The focal areas in the odontogenic ectomesenchymal tissue, particularly near the epithelial elements, showed strong reactivity. Some of the follicular patterns of epithelial elements demonstrated nestin immunoreactivity. Odontoma components showed nestin expression in differentiated odontoblasts, their processes, and adjacent pulp cells. Nestin localisation was also seen in the flat to plump cells adhering the dentinoid matrix and in sparse dentinal fibres in ameloblastic fibro-dentinomas (fig 4).

Ameloblastic fibrosarcoma

Odontogenic ectomesenchymal cells were positive for nestin, although epithelial components were negative in all cases.

AOT

Five of six cases of AOT indicated intense nestin expression in epithelial elements, unlike the odontogenic mixed tumours, which showed immunoreactivity principally in the ectomesenchymal tissue. Nestin was intensely expressed in small nodular areas and in rosette arrangements in the epithelial elements of AOT (fig 5A). These positive cells localised near the droplet extracellular depositions, and haematoxylin and eosin revealed amorphous eosinophilic characteristics of the droplets (fig 5B, C).

Odontogenic fibroma

Of the three cases of odontogenic fibroma, one had odontogenic ectomesenchymal cells that were positive for nestin. This specimen contained abundant odontogenic epithelial strands, and the positive cells presented near the odontogenic epithelium.

Figure 5 (A) AOT displayed intense nestin expression in the scattered nodular foci and rosette arrangements (arrows) of neoplastic tumour cells. (B) Whorled spindle cells in AOT showed strong nestin immunoreactivity. Basophilic amorphous deposits were observed in the tumour cells (arrows). (C) Serial section of (B) stained with haematoxylin and eosin; hyaline droplets were scattered within the tumour (arrows). Original magnification, (A) $\times 2.5$; (B, C) $\times 20$.

Myxoma

Four of the nine cases of myxoma displayed round, angular, or reticular neoplastic cells in the myxoid stroma that were positive for nestin (fig 6). One positive case included

Figure 6 Spindle or reticular tumour cells in the myxoma demonstrated nestin immunoreactivity.

odontogenic epithelial islands within the tumour, but the other eight cases contained no odontogenic epithelial elements.

DISCUSSION

It seems reasonable that some odontogenic tumours express nestin because odontogenic ectomesenchymal tissue is derived from neural crest.^{18–20} However, nestin associated with human odontogenic ectomesenchyme is not expressed throughout life, but rather during limited periods such as odontogenesis and repair of dentine.^{13 15 21} In addition, our characterisation of odontogenic ectomesenchymal tissues indicated no differentiation towards muscle and nervous tissues, and immunohistochemical examination revealed intriguing localisation and distribution of nestin in various odontogenic tumours.

Ameloblastomas and malignant ameloblastomas, which contain no odontogenic ectomesenchyme, did not express nestin, except for one benign case that showed focal nestin localisation in the apical part of the peripheral columnar epithelium of the follicular pattern. In contrast, odontogenic mixed tumours such as ameloblastic fibroma, ameloblastic fibro-odontoma, ameloblastic fibrodentinoma, and ameloblastic fibrosarcoma demonstrated intense expression in the odontogenic ectomesenchymal element, particularly around the neoplastic follicular odontogenic epithelium. In addition, some follicular epithelial components showed nestin localisation in both peripheral ameloblastic columnar cells and inner stellate cells. We also found similar findings in the odontogenic fibroma containing non-neoplastic odontogenic epithelial strands. In human odontogenic tumours, nestin is thought to be expressed in association with mutual interaction between odontogenic epithelium and ectomesenchyme. This is strongly supported by our histological finding that the ectomesenchymal cells around the follicular odontogenic epithelium showed intense reaction and weak or no reactivity further away from the epithelium.

The histogenesis of jaw myxoma is still controversial. Two origins of spindle or stellate tumour cells intermingled within the mucoid matrix have been proposed—that is, dental papilla cells of tooth germ,^{22 23} and mesenchymal cells such as fibroblasts or myofibroblasts other than odontogenic ectomesenchyme.^{24–26} In our study, almost half the cases of jaw myxoma demonstrated nestin immunoreactivity. From the viewpoint of specific nestin immunoreactivity in odontogenic ectomesenchyme, this result implies that there are those two

possible histogeneses of myxoma affecting the jaw. Absence of odontogenic epithelium does not always implicate non-odontogenic origin, as three myxomas without odontogenic epithelium were positive for nestin.

In the odontomas, nestin expression was observed in mature odontoblasts and their processes in the dentinal tubules. The gradient of nestin staining in pulp cells followed differentiation toward the odontoblasts. The same findings were observed in the odontoma-like tissues in the ameloblastic fibro-odontomas. The nestin expression in these lesions may be related to acquisition of the dentine producing function. The phenomenon occurring in odontoma is similar to that in the pulp-odontoblast complex in cases of human dental caries and repair after tooth injury;^{15–21} the dentine matrix is produced in these conditions. Moreover, in the ameloblastic fibrodentinomas, flat or plump cells attached to the dentinoid matrix and their processes in the irregular dentinal tubules were positive for nestin. Although these cells are morphologically different from the mature odontoblasts, the findings suggest that they participate in producing the dentinoid matrix. The rate of nestin positive cases of odontoma was somewhat lower than in other tumours. We consider that nestin expression is under the influence of the differential stages for differentiation. Its expression is lacking in the human completed permanent tooth, as opposed to the tooth germ and developing tooth. The reason for the low positive rate may be that odontoma is composed of differentiated dental components. In fact, some cases, including differentiated odontoblasts and pulp cells mimicking those of intact permanent tooth, did not express nestin.

AOT is not believed to be a true neoplasm, but has varying degrees of inductive change in the connective tissue.¹⁷ Our interesting result for AOT was that nestin was distributed predominantly in the epithelial component. Intense nodular reaction took place in the focal whorled masses of spindle cells and rosette-like structures indicating rows of radial columnar cells, where eosinophilic droplets were found by haematoxylin and eosin staining to be present in the epithelium. AOT is morphologically composed of some types of epithelium. The columnar or spindle epithelium associated with hyaline droplets has a different immunophenotype from other cells.^{27–29} Tatemoto *et al*,³⁰ who investigated localisation

of cytoplasmic skeletal filaments in AOT, emphasised that spindle or columnar epithelium showed very slight reaction with all antibodies to keratins, whereas small and compact cells at the periphery of the focus of spindle or columnar epithelium indicated slight to moderate expression of keratin and vimentin, and showed coexpression. These investigations suggest that the epithelial cells composing AOT are not homogenous. Our study also indicated a heterogeneous pattern of nestin localisation in the AOT. Nestin is supposed to be expressed in association with the production of eosinophilic deposit in the tumour.

The rate of nestin positive cases is variable among odontogenic tumours and jaw myxoma. This may be a result of different origin, such as myxoma, and different stages for differentiation. In addition, it is possibly that the decalcification procedure used may destroy the molecular structure of the antigen. However, we consider that nestin can be regarded as a marker for odontoblasts and odontogenic ectomesenchymal cells in odontogenic tumours.

The supplemental figures can be viewed on the JCP website at <http://www.jclinpath.com/supplemental>.

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- Nestin, a cytoskeleton intermediate filament, is a marker of neural stem cells or progenitor cells, and its expression is also related to tooth development and repair of dentine.
- Nestin expression was immunohistochemically examined using several antibodies on 138 tumours.
- Almost all the ameloblastomas and malignant ameloblastomas were negative for nestin, whereas odontogenic ectomesenchyme in the odontogenic mixed tumours expressed nestin. Nestin was found in neoplastic cells in almost half cases of jaw myxoma and one case of odontogenic fibroma.
- The distribution of nestin in the odontogenic mixed tumours suggests that its expression is upregulated by stimulation from odontogenic epithelium. It may also be involved in the differentiation from pulp cells to odontoblasts in odontogenic tumours.
- Nestin is a useful marker for the odontogenic ectomesenchyme and odontoblasts in odontogenic tumours.

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