Melanocytes in Odontogenic Lesions

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Abstract: Melanocytes are widely distributed in the skin and often present in oral mucosa, but they do not normally exist in bone tissue. Previous reports have shown that melanin pigmentation was seen in some odontogenic lesions of the jaw bone. In this study, the presence of melanin pigmentation and melanocytes was analyzed in ameloblastomas, odontogenic keratocysts (OKCs), and radicular cysts. The aim of this study was to compare the existence of melanin pigmentation and melanocytes in ameloblastomas, radicular cysts, and OKCs, and to clarify the different origins of these odontogenic lesions. Melanin pigmentation was detected using hematoxylin and eosin (HE) staining and Schmorl’s method staining. The presence of melanocytes was confirmed using Melan-A immunohistochemical staining. Melanin pigmentation and melanocytes were shown in OKCs, only melanocytes appeared in ameloblastomas, and neither melanin pigmentation nor melanocytes were present in radicular cysts. Comparing younger and older cases of OKC, both Melan-A and Schmorl’s reaction-positive rates were higher in the younger cases. In conclusion, these data raise the important possibility that the origin of OKC epithelium differs from that of ameloblastoma and radicular cyst based on the expression of melanin pigmentation and melanocytes. These findings also underscore the fact that the origin of OKC differs between younger and older patients. It is suggested that melanin pigmentation and melanocyte expression may help in the classification of OKCs and may be useful in the development of new therapies in the future.

Key words: Melanocyte, Odontogenic keratocyst, Ameloblastoma, Radicular cyst

Introduction

Melanocytes are pigmented-producing cells derived from the neural crest. Melanin pigmentation is widely distributed in the skin and often present in oral mucosa, but normally not present in bone tissue. However, in previous studies, melanin pigmentation was seen in odontomas\(^1,3\), dentigerous cysts\(^4,5\), odontogenic keratocysts (OKCs)\(^6,7\), calcifying odontogenic cysts\(^8,9\), and other odontogenic lesions in the jaw bone. Moreover, the tooth germ originates from the neural crest, but elimination or expression of neural crest cells with odontogenic tissue. However, in previous studies, melanin pigmentation was seen in some odontogenic lesions of the jaw bone. In this study, the presence of melanin pigmentation and melanocytes was analyzed in ameloblastomas, odontogenic keratocysts (OKCs), and radicular cysts. The aim of this study was to compare the existence of melanin pigmentation and melanocytes in ameloblastomas, radicular cysts, and OKCs, and to clarify the different origins of these odontogenic lesions. Melanin pigmentation was detected using hematoxylin and eosin (HE) staining and Schmorl’s method staining. The presence of melanocytes was confirmed using Melan-A immunohistochemical staining. Melanin pigmentation and melanocytes were shown in OKCs, only melanocytes appeared in ameloblastomas, and neither melanin pigmentation nor melanocytes were present in radicular cysts. Comparing younger and older cases of OKC, both Melan-A and Schmorl’s reaction-positive rates were higher in the younger cases. In conclusion, these data raise the important possibility that the origin of OKC epithelium differs from that of ameloblastoma and radicular cyst based on the expression of melanin pigmentation and melanocytes. These findings also underscore the fact that the origin of OKC differs between younger and older patients. It is suggested that melanin pigmentation and melanocyte expression may help in the classification of OKCs and may be useful in the development of new therapies in the future.

Materials and Methods

Study Population

Thirty cases each of ameloblastoma, radicular cyst, and OKC were obtained from the Aichi Gakuin University Affiliated Dental Hospital in Nagoya, Japan. For ameloblastoma cases, 13 specimens were obtained from male patients and 17 from female patients. The age range was 15-79 years, with a mean age of 47.3 years. For radicular cyst cases, 15 specimens were obtained from male patients and 15 from female patients. The age range was 28-81 years, with a mean age of 52.8 years. For OKC cases, 21 specimens were obtained from male patients and 9 from female patients. The age range was 15-78 years, with a mean age of 42.8 years. These OKC cases did not include specimens with BCNS.

All tissues were formalin-fixed and paraffin-embedded. All samples were divided into two groups: the juvenile group and the advanced group. The juvenile group included patients under 30 years of age, and the advanced group included those 30 years of age and older. Among ameloblastoma cases, 6 were in the juvenile group and 24 were in the advanced group. Among radicular cyst cases, 7 were in the juvenile group and 23 were in the advanced group. Among OKC cases, 7 were in the juvenile group and 23 were in the advanced group.

Histopathological examination and special stains

Melanin pigmentation was detected using hematoxylin and eosin (HE) staining and Schmorl’s method. Schmorl’s reaction stain uses the reducing properties of melanin to stain granules blue-green. Potassium hexacyanoferrate (III) (Kanto Kagaku, Tokyo, Japan) and 10% ferric chloride solution (Muto Pure Chemicals Co., Ltd, Tokyo, Japan) were used for Schmorl’s reaction stain, and Kernechtrot stain solution (Muto Pure Chemicals) was used for counterstaining.
Immunohistochemical examination (IHC)

To immunohistochemically confirm the presence of melanocytes, monoclonal mouse anti-human Melan-A (clone A103, M7196; Dako, Glostrup, Denmark) was used according to the manufacturer’s protocol. Mayer’s hematoxylin was used for counterstaining.

Statistical analysis

Statistical analysis was performed using the Chi-squared test. Differences with a p value <0.05 (*) were considered significant, and differences with a p value <0.01 (**) were considered highly significant.

This study was approved by the Ethics Committee of the School of Dentistry, Aichi Gakuin University (approval no. 467).

Results

On HE staining and Schmorl’s method, all ameloblastoma and radicular cyst cases showed negative staining (Fig. 1a, b). In OKC cases, 26.7% (8/30) of specimens were positive for Schmorl’s reaction stain (Figs. 1c, d and 2). The positive rates of males and females were 28.6% (6/21) and 22.2% (2/9), respectively; no significant difference was found between the male and female rates. The positive cases of the juvenile and advanced groups were 85.7% (6/7) and 8.7%
Positive rates of Schmorl’s reaction-positive rates were high in the juvenile group. Comparing juvenile and advanced groups, both Melan-A and Melan-A positive rates were significantly higher in OKCs than in ameloblastomas. These differences were thought to be due to the different migration of melanocytes in tumors and cysts.

In ameloblastomas, Melan-A staining was positive, while Schmorl’s reaction was positive. A highly significant difference was found between juvenile and advanced cases (p<0.01).

On IHC, 3.3% (1/30) of ameloblastoma specimens showed positive Melan-A staining (Figs. 1e and 4). All radical cyst cases did not show positive staining (Fig. 4). In OKC cases, 30.0% (9/30) of specimens were positive for Melan-A (Figs. 1f and 4); a highly significant difference was found between the ameloblastoma and OKC cases (p<0.01). In ameloblastoma cases, the Melan-A positive rates of males and females were 7.7% (1/13) and 0% (0/17), respectively. The positive cases of the juvenile and advanced groups were 16.7% (1/6) and 0% (0/24), respectively. In OKC, the Melan-A positive rates of males and females were 33.3% (7/21) and 22.2% (2/9), respectively; there was no significant difference. The positive cases of the juvenile and advanced groups were 85.7% (6/7) and 13.0% (3/23), respectively (Fig. 5); a highly significant difference was found between juvenile and advanced cases (p<0.01).

Discussion

To the best of our knowledge, this is the first study to compare the distribution of melanin pigmentation and melanocytes in ameloblastomas, radical cysts, and OKCs. Previous studies showed melanin pigmentation in odontomas, dentigerous cysts, OKCs, calcifying odontogenic cysts, and other odontogenic lesions. However, most of these were case reports.

In this study, melanin pigmentation and melanocytes were shown in OKCs, only melanocytes were seen in ameloblastomas, and neither melanin pigmentation nor melanocytes were seen in radical cysts. It can be presumed that these odontogenic lesions have different relationships between their origin and melanocytes.

A radicular cyst is an odontogenic cyst that originates from inflammation with non-vital teeth. The epithelial lining of a radicular cyst derives from proliferation of the epithelial cell rests of Malassez in the periodontal ligament. It has already been reported that melanin pigmentation and melanocytes do not exist in the epithelial cell rests of Malassez. This study showed that neither melanin pigmentation nor melanocytes were present in radicular cysts. These data indicate that radicular cysts originate from the epithelial cell rests of Malassez.

In ameloblastomas, Melan-A staining was positive, while Schmorl’s method was negative. This means that ameloblastomas have only immature melanocytes, with no mature melanocytes. It has been suggested that the growth of melanocytes was inhibited in neoplastic lesions. There have been no reports that ameloblastomas had melanin pigmentation and melanocytes. Ameloblastomas originate from early dental lamina before its calcification. Lawson reported that melanocytes migrate to connective tissue around the tooth germ before calcification. For this reason, a few melanocytes may be present in ameloblastomas.

OKCs were named keratocystic odontogenic tumors and classified as odontogenic tumors in the 2005 WHO classification. Thereafter, OKCs were reclassified as odontogenic cysts in the 2017 WHO classification. Melanin pigmentation was present in OKCs, but not in ameloblastomas. Both OKCs and ameloblastomas originate from early dental lamina before its calcification. In the present study, the frequency of melanocytes was significantly higher in OKCs than in ameloblastomas. These differences were thought to be due to the different migration of melanocytes in tumors and cysts.

In previous studies, melanin pigmentation was observed in patients under 30 years of age. Taken together, OKC samples were divided into juvenile and advanced groups in the present study. Comparing juvenile and advanced groups, both Melan-A and Schmorl’s reaction-positive rates were high in the juvenile group.
These findings indicate that the origin of OKCs differs between younger and older patients. Melanocytes originate from neural crest progenitor cells and reach around the tooth germ connective tissue before the start of calcification of tooth germ\(^\text{12}\). The fact that many melanocytes and melanin pigmentation were observed in the epithelial tissue of the juvenile group may also help explain the origin as the neural crest.

In contrast to the juvenile group of OKC cases, few melanocytes and melanin pigmentation were present in the advanced group cases. It was suggested that epithelial cells of the advanced group originate from such structures as Hertwig’s epithelial sheath and the epithelial remnant of Malassez after tooth eruption, because no melanin pigmentation and melanocytes were reported in the epithelial remnant of Malassez\(^\text{11}\). However, since a few cases in the advanced group of OKC cases were positive for melanocytes and melanin pigmentation, it was considered that some OKCs that occurred at a young age were included in the advanced group cases who had received treatment since becoming older.

In conclusion, the present data raise the important possibility that the origin of OKC epithelium differs from that of ameloblastomas and radicular cysts based on the expression of melanin pigmentation and melanocytes. The present findings also underscore that the origin of OKCs differs between younger and older patients. It is suggested that the epithelial tissue of the juvenile group is considered to be derived from the neural crest, while that of the advanced group originates from such structures as Hertwig’s epithelial sheath and the epithelial remnant of Malassez after tooth eruption. Melanin pigmentation and melanocyte expression may assist in the classification of OKCs and may be useful in the development of new therapies in the future.

One of the limitations of this study is that it was not possible to collect enough samples to achieve a stable outcome. Samples of data from 100 OKCs or more would be necessary. Further studies are needed to clarify the characteristics of melanin pigmentation and melanocytes in odontogenic lesions.

**Conflict of Interest**

The authors have declared that no COI exists.

**References**

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