Expression of PARP 1 in Odontoma

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Abstract: Poly(ADP-ribose) polymerase (PARP 1; EC 2.4.2.30) is a constitutively expressed enzyme that mediates the normal cellular response to DNA damage and interferes with cell differentiation through transcriptional control. PARP 1-deficient mice have been reported to exhibit teeth dysplasia similar to human odontoma. In the present study, we evaluated the contribution of PARP 1 to odontoma development by immunohistochemical staining of human odontoma sections with an anti-PARP 1 antibody. PARP 1 was expressed in odontoblasts, immature enamel and the epithelial component corresponding to ameloblasts. Interestingly, in odontoma, PARP 1 was expressed in the cytoplasm of the ghost cells, which had no nucleus and showed positive staining for cytokeratins. Moreover, the ghost cells showed positive staining with the M30 CytoDEATH antibody, which recognizes a caspase cleavage product of cytokeratin 18 observed in the process of apoptosis, suggesting that some very early events of apoptosis have occurred in these ghost cells expressing PARP 1.

Key words: PARP 1, odontoma, ameloblast, odontoblast, ghost cell

歯牙腫における PARP 1 の発現

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要旨: ポリ(ADP-リボ糖)合成酵素（PARP 1: EC 2.4.2.30）はDNA修復応答や転写制御を介して細胞分化に関与することが知られている。PARP 1欠損マウスではヒト歯牙腫に類似の歯牙異常が認められるという知見がある。本研究では、病理組織学的所見で歯牙腫と診断された標本を用いてPARP 1抗体による免疫染色を行い、PARP 1の発現を調べた。PARP 1は歯牙腫の構成成分において、象牙芽細胞、未熟なエナメル質およびエナメル芽細胞に相当する上皮成分で発現していた。さらに、核を有しないghost cellの細胞質にも発現していた。アポトーシス時にサイトカタチ18がカスパーゼにより切断されるが、ghost cellはこの切断部位を認識するM30 CytoDEATH抗体にも陽性であった。アポトーシスの過程で直接的、間接的な役割を担うPARP 1が発現しているghost cellでアポトーシス初期の現象が起こっていることが示唆された。

索引用語: PARP 1, 歯牙腫, エナメル芽細胞, 象牙芽細胞, ghost cell
Introduction

Poly(ADP-ribose) polymerase (PARP 1; EC 2.4.2.30) is composed of an N-terminal DNA-binding domain, a central regulatory automodification domain that accepts poly(ADP-ribose), and a C-terminal catalytic domain. PARP 1 catalyzes poly ADP-ribosylation of itself and other nuclear proteins using NAD as a substrate. Poly(ADP-ribose) synthesized by PARP 1 was originally found about 40 years ago, and has recently been proposed to be a unique biopolymer associated with many important biological events, such as those in cell viability, cell death and neoplasm formation. PARP 1 modification is known to play roles in chromatin structure formation and consequently alter various gene expressions. Many studies have indicated that PARP 1 is involved in regulating cell differentiation by interfering with transcriptional control. In previous studies, PARP 1-deficient mice were found to be highly susceptible to carcinogenesis induced by alkylating agents and the teeth dysplasia with much irregular odontogenesis similar to human odontoma. Furthermore, changes in PARP 1 expression contributed to cell differentiation during tumor formation, and rearrangements of PARP 1 or differential expression of PARP 1 mRNA have been identified in several cancer cell lines. Moreover, low expression of PARP 1 was found to be correlated with higher genomic instability. Based on these evidences, it is speculated that PARP 1 dysfunction is related to the crisis of the neoplastic lesion.

Some proteins, such as TGFβ-1 and laminin α2, involved in regulating odontogenesis have been identified. Epigenetic controls were also found to contribute to odontoblastic differentiation. This differentiation is characterized by a sequence of events, namely cell cycle withdrawal, cytological polarization and predentin/dentin secretion. However, the mechanisms for human odontoma development have not yet been fully elucidated. Because the abnormality of the differentiation seems to be a cause of the progress of this disease, we evaluated the expression of PARP 1 by immunohistochemical staining of human odontoma sections with an anti-PARP 1 antibody.

Materials and Methods

Samples

Formalin-fixed paraffin-embedded tissues from 7 complex and 6 compound type odontoma cases and 1 calcifying cystic odontogenic tumor associated with odontoma case as a counterpart of the lesion carried the ghost cell were used in this study. The samples were fixed in 10% (v/v) buffered formalin, immersed in the Plank-Rychlo decalcified solution for seven to ten days, embedded in paraffin wax, sectioned and prepared for immunohistochemistry. This study was performed according to the guidelines of the Ethics Committee of Meikai University (A 0313).

Immunohistochemical analysis

The primary antibodies used in this study are listed in Table 1. A biotin-free tyramide signal amplification system (CSAII; DakoCytomation, Kyoto, Japan) was used according to the manufacturer’s instructions. Briefly, deparaffinized sections were immersed in 0.01 M citrate buffer (pH 6.0), heated in a microwave oven for 15 min for antigen retrieval, and then immersed in 3% hydrogen peroxide in phosphate-buffered saline (pH 7.4) for 5 min at room temperature to block endogenous peroxidase activity. Following incubation in 0.25% casein solution for 5 min to block non-specific binding of the primary antibody, the sections were incubated with an appropriately diluted primary antibody for 15 min. Following another incubation in

Table 1 Primary antibodies used for the immunohistochemical analyses.

<table>
<thead>
<tr>
<th>Name</th>
<th>Concentration</th>
<th>Company</th>
<th>Product number</th>
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<tbody>
<tr>
<td>Anti-PARP(214/215)</td>
<td>10 µg/ml</td>
<td>Sigma-Aldrich</td>
<td>P 1991</td>
</tr>
<tr>
<td>Anti-cytokeratin (AE 1/AE 3)</td>
<td>2.7 µg/ml</td>
<td>DakoCytomation</td>
<td>M 3515</td>
</tr>
<tr>
<td>M30 CytoDEATH</td>
<td>6.6 µg/ml</td>
<td>Roche</td>
<td>2 140 322</td>
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0.25% casein solution for 5 min to block non-specific binding of the secondary antibody, the sections were sequentially incubated in a horseradish peroxidase (HRP)-conjugated secondary antibody for 15 min, followed by FITC-labeled tyramide for 15 min. The deposited FITC-labeled tyramide was detected by incubating the sections with an HRP-labeled anti-FITC antibody for 15 min. The deposited FITC-labeled tyramide was detected by incubating the sections with an HRP-labeled anti-FITC antibody for 15 min. Visualization of HRP was carried out using the hydrogen peroxide/3,3’-diaminobenzidine tetrahydrochloride reaction for 5 min, followed by counterstaining of the nuclei with Mayer’s hematoxylin. The stained sections were analyzed using a microscope equipped with a CCD camera (Olympus BX 33-S; Olympus Corporation, Tokyo, Japan).

**Results**

**Immunostaining with an anti-PARP 1 antibody**

Immunostaining of samples with the anti-PARP 1 antibody is summarized in **Table 2**. Odontoma samples of both the complex and compound types exhibited that PARP 1 was expressed in odontoblasts, immature enamel, the epithelial component corresponding to ameloblasts and the ghost cells (**Table 2**). PARP 1 expression was identified in odontoblasts (**Fig 1a**). A subset of the cells that corresponded to dental pulp of the lesion was positively stained (**Fig 1a**). Immature enamel also showed positive staining for PARP 1 (**Fig 1b**), whereas mature dentin was clearly negative (**Fig 1a, b**). The epithelial component corresponding to ameloblasts showed positive staining for PARP 1 (**Fig 1c**).

**Characterization of ghost cells expressing PARP 1.**

Non-nucleated ghost cells showed positive staining

<table>
<thead>
<tr>
<th>Sample</th>
<th>Positive staining</th>
<th>Positive cells</th>
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<tbody>
<tr>
<td>Odontoma Complex</td>
<td>7</td>
<td>Odontoblasts</td>
</tr>
<tr>
<td>Compound</td>
<td>6</td>
<td>Immature enamel</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Epithelial component</td>
</tr>
<tr>
<td></td>
<td></td>
<td>corresponding to ameloblasts</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ghost cells</td>
</tr>
</tbody>
</table>

**Fig 1** Localization of PARP 1 in odontoma sections. (a) Expression of PARP 1 in odontoblasts (arrows). P: dental pulp; mD: mature dentin. (b) Expression of PARP 1 in immature enamel of an odontoma. imE: immature enamel; mD: mature dentin. (c) Expression of PARP 1 in the epithelial component corresponding to ameloblasts (arrows). Scale bar: 100 µm.
with eosin (Fig 2a) and for cytokeratins AE 1 and AE 3 (Fig 2b). PARP 1 was expressed in the ghost cells in odontoma (Fig 2c). Moreover, the ghost cells revealed positive staining with the M30 CytoDEATH antibody against a caspase cleavage product of cytokeratin 18 (Fig 2d).

**Discussion**

In the present study, we first showed that PARP 1 is expressed in constituents of odontoma such as odontoblasts, immature enamel and the epithelial component corresponding ameloblasts, suggesting that PARP 1 is possibly involved in the formation of odontoma. Based on many reports on the function of PARP 1, we speculate that the differential expression of PARP 1 can modify to the cell differentiation of the odontoma, although the pathogenesis of the tumor remains to be elucidated.

PARP 1 is a target of the caspase protease activity associated with apoptosis. Although the role of PARP 1 in apoptosis remains to be elucidated, PARP 1 cleavage is considered to be a marker of apoptosis. Some previous data have suggested that PARP 1 activation is a signal for the release of apoptosis-inducing factor (AIF) from mitochondria, resulting in programmed cell death via a caspase-independent pathway.

Interestingly, we found that PARP 1 is also expressed in ghost cells in odontomas. To confirm that the cells expressing PARP 1 were actually ghost cells,
Expression of PARP 1 in odontoma

we examined the expression of cytokeratins AE 1/AE 3 in the cells, since there is evidence that stronger staining with cytokeratins AE 1/AE 3 is prevalent in ghost cells\(^1\). Ghost cells or their analogue, shadow cells have been also found in adamantinomatous cranioparyngioma, pilomatrixoma and calcifying cystic odontogenic tumors, as well as in complex and compound odontomas. In present study, a calcifying cystic odontogenic tumor associated with odontoma was examined as a control, and the ghost cells in this tumor were shown to express PARP 1 (data not shown). The nucleated cells adjacent to ghost cells expressed Bax, but not Bcl-2 or Bcl-X\(_{L}\). Moreover, some cells around ghost cells showed positive staining with the TUNEL assay\(^2\). In another study, Mib-1 and bcl-2 showed strong positivity in cells of the odontogenic epithelium, but were completely negative in the ghost cells\(^3\). The immunohistochimical studies have indicated that ghost cells may be an abnormal form of keratinization\(^2,3\). We found that the ghost cells were positively stained with an antibody against a caspase cleavage site within cytokeratin 18. Keratins, in particular cytokeratin 18, are affected during the early events of apoptosis\(^4,5\).

Taken together, these findings indicate that the ghost cells may undergo abnormal terminal differentiation as an apoptotic process. This apoptotic process separates the amino-terminal DNA-binding domain of PARP 1 from the C-terminal catalytic domain, resulting in the loss of normal PARP 1 function. We propose the hypothesis that loss of function of PARP 1 could contribute to the development of odontoma, supported by an evidence that PARP 1-deficient mice showed the abnormality of the odontogenesis such as much irregular enamel and dentin\(^6\). To elucidate the mechanism of the disordered differentiation in odontogenesis, in which PARP 1 is involved, further analyses using PARP 1-deficient mice are necessary.

References

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