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Short Communication

Predentin's influence on clastic cell behavior in human external cervical resorption: Evidence from a case study

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Abstract External cervical resorption (ECR) is an aggressive disease characterized by resorption of the tooth root structure. While the pericanalar resorption-resistant sheet (PRRS) impedes ECR progression towards the pulp, the underlying mechanisms of its protective role in human teeth remain unclear. This study aimed to elucidate the pathology of ECR in a 31-year-old female patient by employing radiographic, histological, and immunohistochemical analyses of an extracted tooth. Histological examination revealed that the PRRS comprised dentin, predentin, and reparative bone-like tissue. Notably, clastic cells were observed on the surfaces of all three tissues within the same specimens. Immunohistochemical staining for cathepsin K demonstrated diminished resorptive activity of clastic cells on predentin compared to dentin and bone-like tissue. These findings suggest a potential role for predentin in attenuating clastic cell activity, potentially serving as the final barrier safeguarding the pulp tissue.

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Introduction

External cervical resorption (ECR) remains the most enigmatic form of external root resorption, characterized by the progressive destruction of the tooth root via clastic cell activity originating from the periodontium.[1] This complex process involves the initial breakdown of cementum, enabling direct interaction between clastic cells and dentin.[1] Subsequently, ECR progresses by invading the dentin towards the pulp, potentially compromising pulp vitality. However, the pericanalar resorption-resistant sheet (PRRS) serves as a critical barrier, impeding ECR from perforating into the root canal and preserving pulp health.[2] Morphological evaluations in human teeth have confirmed the presence of the PRRS, a structure demonstrably composed of predentin, dentin, and occasionally bone-like tissue.[2] Histological analysis further reveals the presence of multinucleated clastic cells on the surface of the PRRS.[2] While predentin adjacent to the pulp is suspected to play a pivotal role in hindering ECR progression towards the root canal, the underlying mechanisms remain elusive. Osteoclasts are known to adhere to arginine—glycine—aspartate (RGD) sequences on the extracellular matrix, facilitating their resorptive activity.[3] Importantly, predentin and precementum lack these RGD sequences, reducing clastic cell binding and activity, thereby contributing to root resorption resistance and protecting the pulp tissue.[4] While *in vitro* studies conducted in the 1980s and 1990s support this concept,[5] evidence from human ECR samples and comparisons of clastic cell activity on different PRRS components (predentin, dentin, and bone-like tissue) are currently lacking.

To bridge this knowledge gap, this study delved into the histological characteristics of human ECR teeth and undertook a comparative analysis of clastic cell activity across the distinct tissue components of the PRRS in an extracted tooth from a 31-year-old female patient.

Materials and methods

Micro-computed tomography (Micro-CT)

A 31-year-old Japanese woman with no significant medical history presented to our clinic, complaining of dull pain during mastication in her left mandibular second molar (tooth #37). The tooth was diagnosed with external cervical resorption. Following this diagnosis, the patient opted for autologous tooth transplantation using a wisdom tooth. Consequently, tooth #37 was extracted. The tooth was fixed with 10% neutral buffered formalin and imaged using a SkyScan 1176 X-ray micro-CT system (Bruker, Kontich, Belgium). The operating settings for the X-ray source were set to 50 kVp and 500 μA. The image reconstitution was performed using NRecon software (Bruker). After reconstruction, Three-dimensional (3D) image processing and analysis were performed using the DataViewer and CTAn software (Bruker).

Tissue preparation, histology, and immunohistochemistry

The fixed tooth was decalcified with K-CX (Falma, Tokyo, Japan) for 7 days and embedded in paraffin. The paraffin-embedded tissue blocks were cut into 4-μm-thick sections for hematoxylin and eosin (H&E) and immunohistochemical staining. Immunostaining was performed using an EnVision/horseradish peroxidase (HRP) kit (DAKO-Agilent Technologies Co., Santa Clara, CA, USA). Briefly, the sections were treated with a 0.1% hydrogen peroxide-methanol solution to inhibit endogenous peroxidase activity and 5% bovine serum albumin/Tris-buffered saline (TBS) to block non-specific binding of primary antibodies. Subsequently, each section was incubated with the primary antibody against cathepsin K (ab19027, Abcam, Cambridge, UK, 1:100 dilution) or CD68 (DAKO, Ready-to-use) at 4 °C overnight. These sections were then incubated with HRP-conjugated polymer anti-rabbit or anti-mouse antibodies. The peroxidase activity was visualized using 0.1% 3, 3′-diaminobenzidine and 0.01% hydrogen peroxide in TBS. The images of H&E and immunohistochemical staining were captured using an AXIO Vert. A1 microscope (Carl Zeiss Inc., Oberkochen, Germany). The images were processed using ZEN 2010 B Sp1 Ver. 6.0.0.485 software (Carl Zeiss).

Results

Macroscopic examination of the extracted tooth (#37) with ECR from a 31-year-old female patient revealed a soft tissue filling the root resorption site on the distal aspect. This tissue resembled gingiva and fibrous connective tissue (Fig. 1A and B). Micro-CT analysis was performed to further characterize the resorption defect. Three-dimensional reconstructions revealed that the initial point of entry (portal of entry) was located at the cemento-enamel junction, extending from the distal to buccal aspects. Dentin resorption was predominant, with minimal involvement of the enamel (Fig. 1A', B′). Distal micro-CT images and 3D reconstructions demonstrated extensive internal dentin resorption surrounding the pulp canal space. Notably, the defect cavity itself was considerably larger than the relatively small entry (Fig. 1C, C′). In axial micro-CT images, a separation wall (i.e., the PRRS) was observed between the pulp canal space and resorption margin (white arrow, Fig. 1D, D'). During specimen preparation, the tooth was sectioned in the mid-defect region with an axial orientation (Fig. 1E). H&E staining of the sections revealed histologically normal pulp tissue within the root canal (Fig. 1F—I). Additionally, dilated blood vessels (hyperemia) were observed on the side adjacent to the ECR (Fig. 1J-L). These results suggest no evidence of pulp necrosis or bacterial infection.

In this case, root resorption by ECR progressed through the dentin towards the pulp and eventually reached the predentin layer. Lingual resorption area was predominantly occupied by reparative bone-like tissue (yellow dotted line)

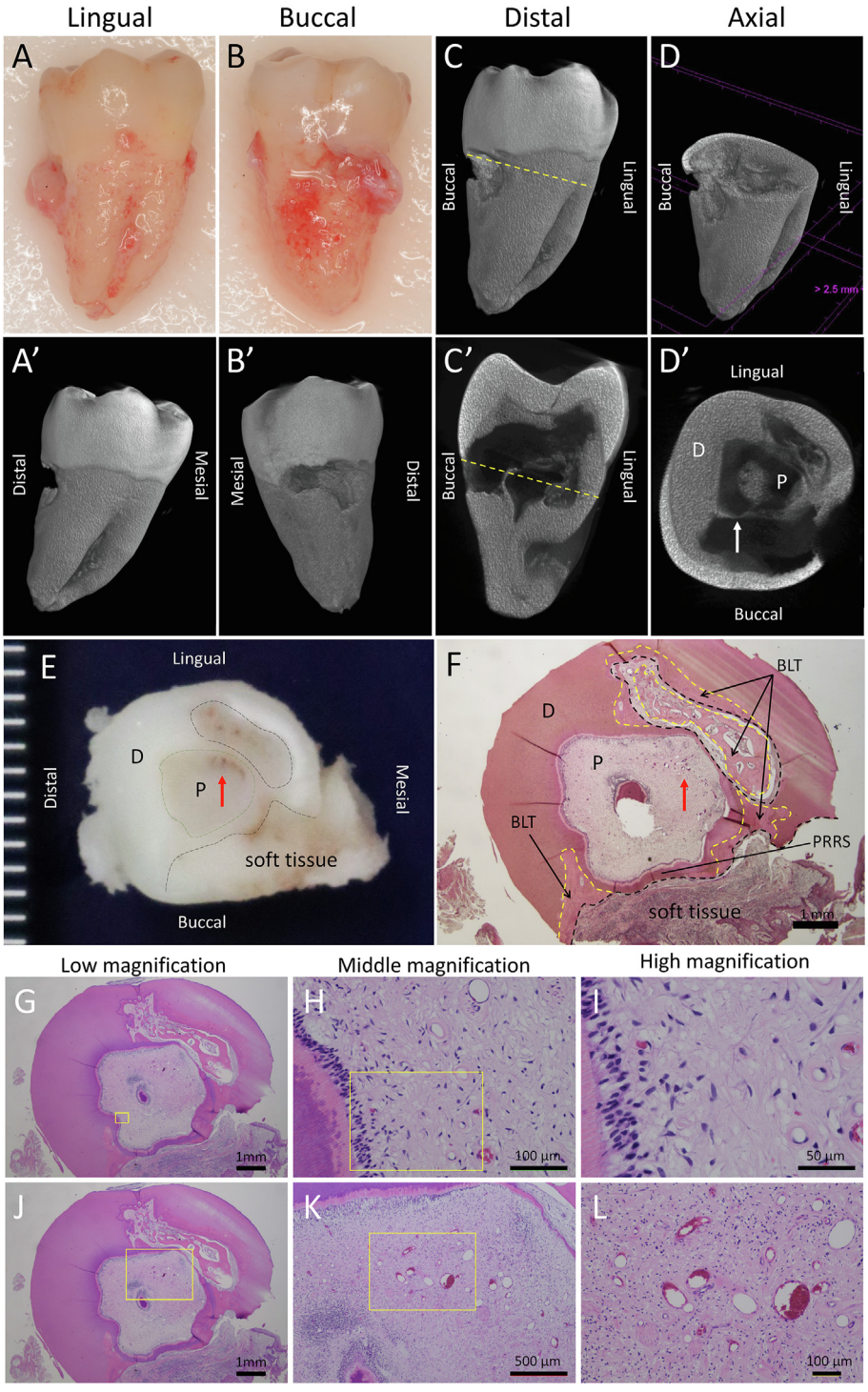

Figure 1 Micro-CT and histological analysis of the extracted tooth with external cervical resorption. Photographs and 3D reconstructed micro-CT images of the extracted mandibular second molar #37 (A, A′) on the lingual side and (B, B′) on the buccal side. (C) Three-dimensional micro-CT image of the distal side and (C′) coronal micro-CT image. The yellow dotted line indicates the height of the axial section. (D) Axial 3D and (D′) 2D micro-CT images. The white arrow indicates the pericanal resorption-resistant sheet. (E) Mandibular second molar #37 is cut in the middle of the defect in an axial section, and (F) specimen of an axial section is subjected to H&E staining. The distal resorption area is filled with fibrous connective tissue. Lines indicate pulp area (green dotted line), resorption area (black dotted line), and bone-like tissue (yellow dotted line). The red arrow indicates the hyperemia region. (G) Low-magnified, (H) middle-magnified, and (I) high-magnified H&E staining images of normal pulp tissues. (J) Low-magnified, (K) middle-magnified, and (L) high-magnified H&E staining images of hyperemia. Yellow boxes indicate the areas of higher magnification images. Abbreviations: P, pulp; D, dentin; BLT, bone-like tissue; PRRS, pericanal resorption-resistant sheet. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

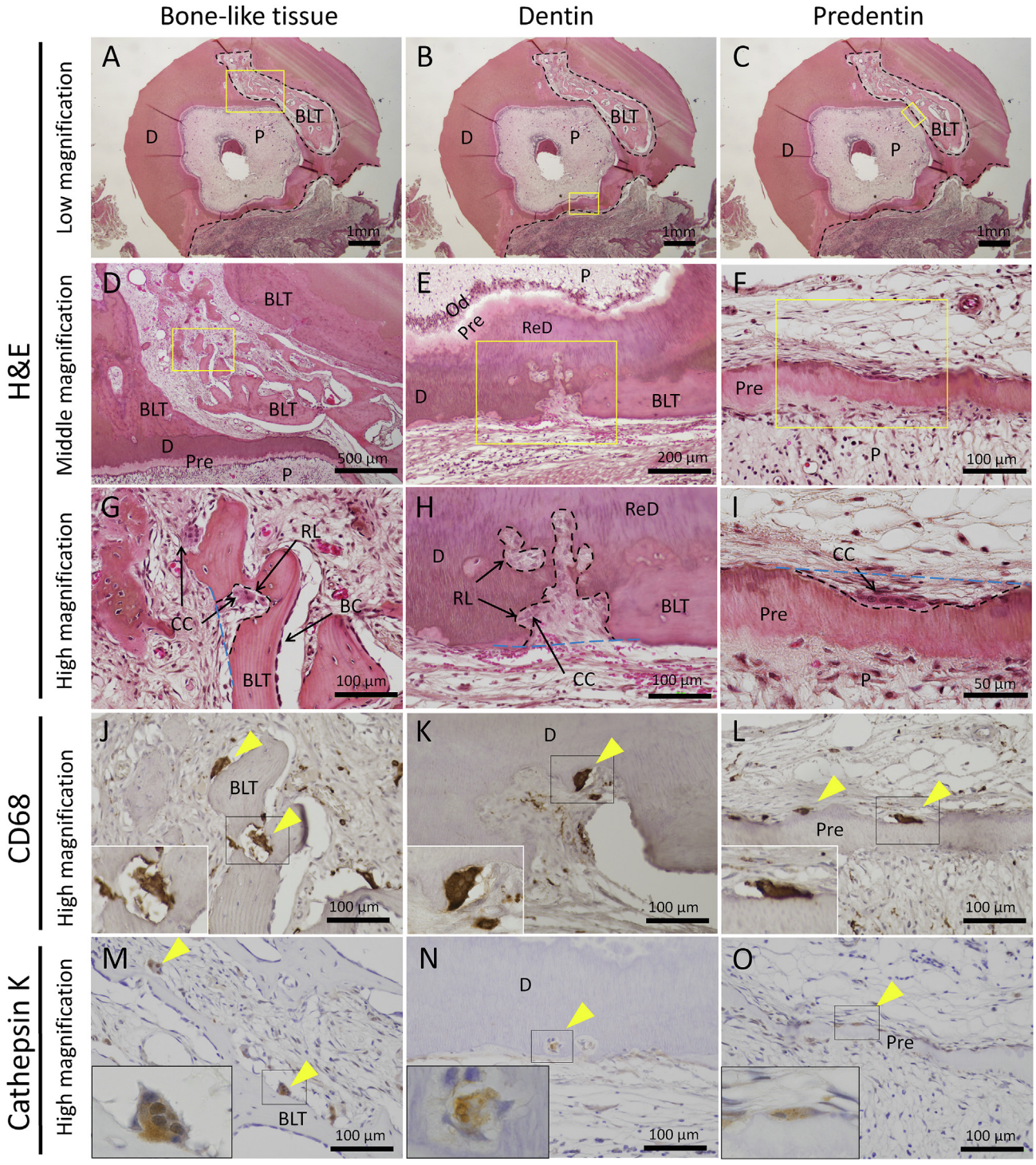

Figure 2 Histological and immunohistochemical analyses of the extracted tooth with external cervical resorption. Axial H&E staining at low magnification. The yellow rectangle indicates areas containing (A) bone-like tissue, (B) dentin, and (C) predentin. Middle-magnification images of (D) bone-like tissue, (E) dentin, and (F) predentin. High-magnification images of the (G) bone-like tissue, (H) dentin, and (I) predentin. Dotted lines indicate the surface of tissues (blue) and erosion area (black). Immunohistochemical staining for CD68 was performed and photographed at high magnification in the (J) bone-like tissue, (K) dentin, and (L) predentin. Immunohistochemical staining for cathepsin K in the (M) bone-like tissue, (N) dentin, and (O) predentin. Yellow arrows indicate multinucleated clastic cells. The black rectangle shows the area of the magnified image in the lower-left corner. Abbreviations: P, pulp; Od, odontoblastic layer; Pre, predentin; D, dentin; BLT, bone-like tissue; CC, clastic cells; BC, blast cells; RL, resorption lacunae; ReD, reparative dentin. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(Fig. 1F). Subsequently, the resorption sites in the reparative bone-like tissue (Fig. 2A), dentin (Fig. 2B), and predentin (Fig. 2C) were compared using identical specimens. The reparative bone-like tissue harbored osteocytes and exhibited the characteristics similar to those of a typical trabecular bone. Consequently, it was readily distinguished from dentin (Fig. 2D). At the advanced dentin resorption site near the pulp, reparative processes were evident: bone-like tissue and reparative dentin (Fig. 2E). Despite aggressive dentin resorption, the predentin adjacent to the pulp tissue exhibited minimal resorption by clastic cells (Fig. 2F). This suggests a potential protective role of the predentin against pulp damage. High-magnification images revealed the morphology of multinucleated clastic cells and erosion depth of the resorption lacunae. Clastic cells on the bone-like tissues and dentin displayed a relatively round morphology (Fig. 2G and H), while those on the predentin appeared flattened (Fig. 2I). As previously reported by Eriksen et al., erosion depth serves as a reliable indicator of clastic activity.[6,7] The erosion depth in the bone-like tissues and dentin was deep, forming tunnel-like structures (Fig. 2G and H). Conversely, the erosion depth in the predentin remained shallow (Fig. 2I).

To further characterize the clastic cells, immunohistochemical staining for CD68 was performed.[8] Clastic cells throughout all examined tissues exhibited uniform CD68 positivity, irrespective of the underlying substrate (Fig. 2J-L). Notably, CD68-positive cells on the bone-like tissue and dentin displayed deeper penetration into the resorption lacunae (Fig. 2J and K), compared to those on the predentin, which only contacted the tissue surface (Fig. 2L). Subsequently, immunohistochemical staining for cathepsin K was employed to assess clastic cell activity.[9] Clastic cells within the reparative bone-like tissues and dentin demonstrated strong cathepsin K expression, indicative of highly active resorption (Fig. 2M and N). Conversely, clastic cells on the predentin exhibited lower cathepsin K expression levels (Fig. 2O). This finding suggests a potential attenuation of clastic activity as these cells approach the predentin layer.

Discussion

Our findings demonstrated that clastic cells on bone-like tissue and dentin exhibited robust expression of cathepsin K, while those on predentin displayed significantly reduced cathepsin K expression (Fig. 2).

Clastic cell-mediated resorption is initiated by a two-step adhesion process. Initially, clastic cells bind to the surface via non-$\alpha v\beta 3$ integrins and utilize cathepsin K to perform limited proteolytic matrix degradation exposing cryptic RGD motifs.[10] Subsequently, $\alpha v\beta 3$ integrins on the clastic cell surface recognize and bind to these exposed RGD sequences, leading to the formation of a sealing zone and an actin ring. This tight adhesion facilitates the release of abundant cathepsin K into the resorption lacuna, promoting extensive degradation of the collagen-rich matrix.[10] *In vitro* studies suggest that clastic cells attach to predentin but fail to spread, indicating its resistance to resorption. This resistance is likely attributed to the absence of RGD motifs in predentin, hindering clastic cell binding and activity.[3,4] Our findings in human samples further support this hypothesis. Clastic cells associated with the predentin exhibit lower cathepsin K expression and display a morphology incompatible with active resorption (Fig. 2). These observations suggest that the lack of RGD sequences in predentin prevents clastic cells from forming a sealing zone and actin ring, thereby hindering their transition to the active resorption phase.

The results of the present study suggest that the predentin may possess inherent mechanisms to resist resorption. These findings can potentially facilitate further elucidation of the influence of predentin on clastic cell behavior. Additionally, they may provide valuable insights for clinicians in developing diagnostic and treatment strategies.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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