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Pseudoepitheliomatous Hyperplasia Associated with Bisphosphonate-related Osteonecrosis of the Jaw

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Abstract. *Bisphosphonate-related osteonecrosis of the jaw (BRONJ) is a well-characterized oral complication of systemic therapy with bisphosphonates. Pseudoepitheliomatous hyperplasia was observed in some of the lesions. Because podoplanin expression has been linked to malignant lesions of the oral mucosa, we aimed to investigate podoplanin expression in the pseudoepitheliomatous hyperplasia. We analyzed archival paraffin- and plastic-embedded specimens from BRONJ using both conventional and immunohistochemical (AE1/AE3, D2-40) staining methods. Eleven out of seventeen BRONJ cases showed pseudoepitheliomatous hyperplasia. All these cases were positive for AE1/AE3 and pseudoepitheliomatous hyperplasia displayed a strong basal and parabasal reaction against podoplanin. The podoplanin expression in pseudoepitheliomatous hyperplasia in BRONJ specimens should not be considered a sign of malignancy. We discuss the current and possible future roles of surgical pathologists in diagnosing morphological changes associated with the development and therapy of BRONJ lesions.*

Ten years ago, Robert E. Marx first described a series of patients suffering from exposed and non-healing jaw bones after treatment with bisphosphonates, a side-effect subsequently confirmed by several other studies and known as bisphosphonate-associated osteonecrosis of the jaw (BRONJ) (1). Although several hypotheses regarding the pathogenesis of BRONJ have been proposed, the exact mechanism of bisphosphonate-associated osteonecrosis has not yet been determined. According to the definition of BRONJ established by the American Association of Oral and Maxillofacial Surgeons (AAOMS), patients may be considered to have BRONJ if each of the following three characteristics is present: (1) Current or previous treatment with a bisphosphonate; (2) exposed, necrotic bone in the maxillofacial region that has persisted for more than eight weeks; and (3) no history of radiation therapy to the jaws (2). Even though initial stages of BRONJ do not require intervention other than periodic antibiotic oral rinses, sometimes in combination with systemic antibiotic therapy, the large burden of necrotic bone results in extra-oral fistulization (3, 4), pathological fracture or progressive development of an oral-maxillary sinus fistula which require for aggressive surgical management.

Microscopically, advanced lesions of BRONJ are characterized by findings of bacteria (4-9), necrotic bone sequestra, purulent inflammation, and granulation tissue with numerous osteoclasts (10-13) associated with sclerotic changes of remaining viable bone tissue (10, 14). Furthermore, a pseudoepitheliomatous hyperplasia (4) has been observed in bisphosphonate osteonecrosis of the jaw (BRONJ) lesions.

Podoplanin (synonyms: T1α, aggrus, human gp36) is a type-1 transmembrane sialomucin-like glycoprotein consisting of 162 amino acids, nine of which are form the intracellular domain (15). The extracellular domain of podoplanin is rich in Ser and Thr and contains multiple potential O-glycosylation sites (16). As podoplanin is expressed in lymphatic but not in blood vessel endothelium, it has been widely used as a specific marker for lymphatic endothelial cells and lymphangiogenesis (17-19). Recent studies, however, have described podoplanin expression in various normal and neoplastic tissues. Even though podoplanin expression has been reported in both neoplastic (20) and inflammatory altered oral mucosa (21) as well as in odontogenic lesions (22-26), normal oral mucosa does not exhibit podoplanin expression with the exception of the submucosal lymph vessels (21).

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Because podoplanin expression has previously been reported in inflamed gingival epithelium (21), we hypothesized that podoplanin expression is enhanced in pseudoepitheliomatous hyperplasia in BRONJ. In the present study, we therefore investigated (a) the occurrence of pseudoepithelial hyperplasia in our cases of partial jaw bone resection specimens with BRONJ, and (b) the expression of podoplanin in cases showing pseudoepithelial hyperplasia.

Materials and Methods

Case selection. We retrospectively investigated morphological changes in cases of BRONJ with partial jaw bone resection treated at the University Medical Center Hamburg-Eppendorf, Germany from April 2011 to April 2013. Each patient was evaluated clinically and additional imaging examinations were performed. All patients fulfilled the AAOSM criteria of BRONJ (2).

Immunohistochemical staining and evaluation. The archival tissues were paraffin- and methyl-methacrylate-embedded bone specimens from each case, which were stained with Goldner trichrome, periodic acid-Schiff, Giemsa and haematoxylin and eosin staining methods. All slides were reviewed, and representative sections from the formalin-fixed, paraffin-embedded tissue blocks were examined using automated immunohistochemistry systems. The freshly-cut sections were loaded into a PT Link module (Dako, Glostrup Denmark) and subjected to an antigen retrieval/de-waxing protocol with the Dako EnVision FLEX Target Retrieval Solution, at high pH, and then transferred to the Dako Autostainer Link 48 instrument. Immunostaining was performed using the primary antibodies AE1/AE3 (Dako IR053, Glostrup, Denmark), and D2-40 (Dako IR072, Glostrup, Denmark), and the Dako EnVision Flex detection system. Microscopic analyses were performed using a Zeiss microscope (Axiophot, Carl Zeiss, Jena, Germany) and representative microphotographs were performed using a digital camera (AxioCam MRc, Carl Zeiss, Jena, Germany), and AxioVision Rel.4.8 imaging software (Carl Zeiss, Jena, Germany). Both immunohistochemical staining intensity (scored as negative, weak, moderate or strong) and distribution (basal, parabasal, luminal) were recorded along with the relative proportion of immunohistochemically-positive cells.

Results

Study cohort demographics. The study cohort consisted of 17 consecutive patients who underwent a partial jaw resection for BRONJ at the University Medical Center Hamburg-Eppendorf, Germany from April 2011 to April 2013. Demographic and clinical data are summarized in Table I.

Morphological characteristics of BRONJ lesions. All specimens showed a similar layering structure in the BRONJ lesions. Clinical radiographs and computed tomography (Figure 1A) showed an osteolytic jaw bone lesion with central sequester. The gross analysis of the resection specimens showed periostal bone formation (Figure 1B) and central necrotic tissues. The specimens were verticaly-lamellated and further analyzed radiographically and macroscopically. The specimens displayed jaw bone with new periostal bone formation and a central osteolytic lesion reaching the oral surface. On slab radiographs, osteolysis was seen to reach both the cortical bone and the mineralized periostal bone (Figure 1C) in the majority of our cases. The osteolytic bone defects contained of soft tissue with scattered fragments of necrotic bone sequestrae. Macroscopically (Figure 1D), the superficial necrotic areas appeared red to brown colored, depending on the degree of formalin fixation of the tissues. The remaining cortical bone appeared somewhat more yellowish when compared to intraosseous sclerotic spongiosa and newly-periostal formed bone tissue. None of the cases from our study cohort showed extraoral fistulization.

Microscopically, the superficial areas on the BRONJ lesions contained necrotic bone colonized by diverse bacteria (Figure 2A) including characteristic colonies of Actinomyces (Figure 2B). The latter was found both in-between necrotic trabeculae and also attached to the trabecular surface. Six out of seventeen cases showed areas of de-mineralization of the necrotic bone sequester, partially superficial (Figure 2C), but also deeper in the bone matrix (Figure 2D). Some necrotic bone particles were embedded in the inflamed granulation tissue and were closely-associated with the pseudoepitheliomatous hyperplasia (Figure 2E). Other bone lesions showed continuous superficial pseudoepitheliomatous hyperplasia with extensions into the deeper layers and massive purulent exudate (Figure 2F). Some of the lesions displayed deep viable bone tissue covered by inflamed granulation tissue lined-up with pseudoepithelial hyperplasia (Figure 2G). Numerous osteoclasts were apparent within the granulation tissue and on the border of the viable bone remnant as well (Figure 2H). The majority of the resection specimens showed distinct new periosteal bone formation (Figure 2I).

Pseudoepitheliomatous hyperplasia and podoplanin expression in clinical samples. Pseudoepitheliomatous hyperplasia was observed in 11 out of 17 BRONJ cases (Table I) in our study cohort and consisted of irregular squamous epithelial proliferation (Figure 3A) associated with inflammation and formation of granulation tissue. The pseudoepithelial hyperplasia was one to several cell layers-thick and located both at the bone surface with extension into the granulation tissue and on the inner surface of the BRONJ lesion. All cases showed positive immunohistochemical reaction against the pancytokeratin marker AE1/AE3 (Figure 3B) and positive reaction of basal and parabasal keratinocytes with podoplanin (Figure 3C), as well. Even though mitoses were found within the pseudoepithelial hyperplasia, no dysplastic changes in the epithelial cells were observed.

Table I. *Bisphosphonate-related osteonecrosis of the jaw. Characteristics of the study cohort.*

	Age/Gender	Primary diagnosis	Bisphosphonate (months)	Duration of medication hyperplasia	Pseudoepithe-liomatous (full thickness)	AE1/AE3 (epithelial cells)	D2-40
1	63/male	Prostate cancer	Zolendronate *i.v.*	73	-	-	-
2	80/male	Prostate cancer	Zolendronate *i.v.*	49	Present	Strong reaction	Basal/parabasal
3	89/male	Prostate cancer	Zolendronate *i.v.*	47	Present	Strong reaction	Basal/parabasal
4	75/male	Prostate cancer	Zolendronate *i.v.*	28	Present	Strong reaction	Basal
5	62/male	Renal cancer	Zolendronate *i.v.*	33	-	-	-
6	82/male	Multiple myeloma	Zolendronate *i.v.*	38	Present	Strong reaction	Basal/parabasal
7	71/male	Prostate cancer	Zolendronate *i.v.*	51	-	-	-
8	65/male	Prostate cancer	Zolendronate *i.v.*	54	-	-	-
9	73/female	Osteoporosis	Alendronate *p.o.*	107	Present	Strong reaction	Basal/parabasal
10	78/male	Prostate cancer	Zolendronate *i.v.*	73	Present	Strong reaction	Basal
11	60/female	Osteoporosis	Alendronate *p.o.*	121	-	-	-
12	54/female	Uterine cancer	Zolendronate *i.v.*	13	-	-	-
13	62/male	Multiple myeloma	Zolendronate *i.v.*	30	Present	Strong reaction	Basal/parabasal
14	74/female	Lung cancer	Zolendronate *i.v.*	49	Present	Strong reaction	Basal
15	78/female	Breast cancer	Zolendronate *i.v.*	96	Present	Strong reaction	Basal
16	66/female	Osteoporosis	Alendronate *p.o.*	128	Present	Strong reaction	Basal/parabasal
17	77/female	Breast cancer	Zolendronate *i.v.*	57	Present	Strong reaction	Basal/parabasal

Discussion

Bisphosphonate-related osteonecrosis of the jaw (BRONJ) is a well-characterized oral complication that affects approximately 0.94%-10% of patients with cancer who are treated with intravenous bisphosphonates (27, 28). It is rare in patients taking oral bisphosphonates for osteoporosis. In the vast majority of cases, BRONJ is characterized by exposed necrotic bone in the oral cavity that develops spontaneously or following dental extractions or other surgical procedures (29); however, cases with non-exposed osteonecrosis have also been reported (30, 31).

Our study confirmed the finding of a characteristic layering structure of BRONJ lesions (4, 10) with superficial necrotic bone tissue colonized by bacteria, deeper inflamed granulation tissue and sclerotic bone and focal new periostal bone formation. Even though most researchers (3-5, 11, 12, 14, 32, 33) investigated decalcified bone tissues taken from BRONJ lesions, few studies of non-decalcified bone biopsies from BRONJ lesions have already been reported (11, 34, 35). We also chose an undecalcified method of processing bone biopsies, because it enabled for light microscopic analysis of both non-mineralized and mineralized bone matrix. Interestingly, we observed de-mineralization of necrotic bone tissue in BRONJ lesions that did not appear to be linked with osteoclastic bone resorption. Areas with newly-formed osteoid rimmed by osteoblasts and mineralized new periostal bone formation were also well-demonstrated using undecalcified bone specimens. In contrast to Bedogni *et al*. who suggested osteomalacia in patients with BRONJ (35), we observed reactive new periosteal bone formation and maturing callus tissue in the jaw bone. However, further research on local and systemic bone metabolism in BRONJ patients is necessary to explain for the possible causal relationship between adverse effects of bisphosphonate medication and osteomalacia.

Similarly to Hansen *et al*. (4, 32), we observed pseudoepithelial hyperplasia in a substantial proportion of BRONJ cases and can confirm their suggestion that it is a relatively common feature reflecting the oral mucosal disruption. As our study cohort did not contain cases with extraoral fistulization, we suggest that the pseudoepithelial hyperplasia either arose from remaining periodontal squamous epithelium or represents epithelial proliferation of the neighboring gingival epithelium rather than ingrowths of the squamous epithelium of the skin. We hypothesize that this finding represents an attempt of the oral tissues to demarcate the necrotic inflamed tissues and to subsequently re-epithelialize the bottom of the ulcerated BRONJ lesions and thus restore mucosal barrier function. This suggestion was further supported by the finding of podoplanin expression by basal and parabasal keratinocytes, analogous to the staining pattern reported in chronic inflamed gingival epithelium (21).

In the current study, we observed podoplanin expression in the basal and parabasal epithelial layers in pseudopeitheliomatous hyperplasia associated with BRONJ. Even though podoplanin has been linked to oral squamous cancer (20, 36-42), the pattern of its expression in pseudoepitheliomatous hyperplasia associated with BRONJ

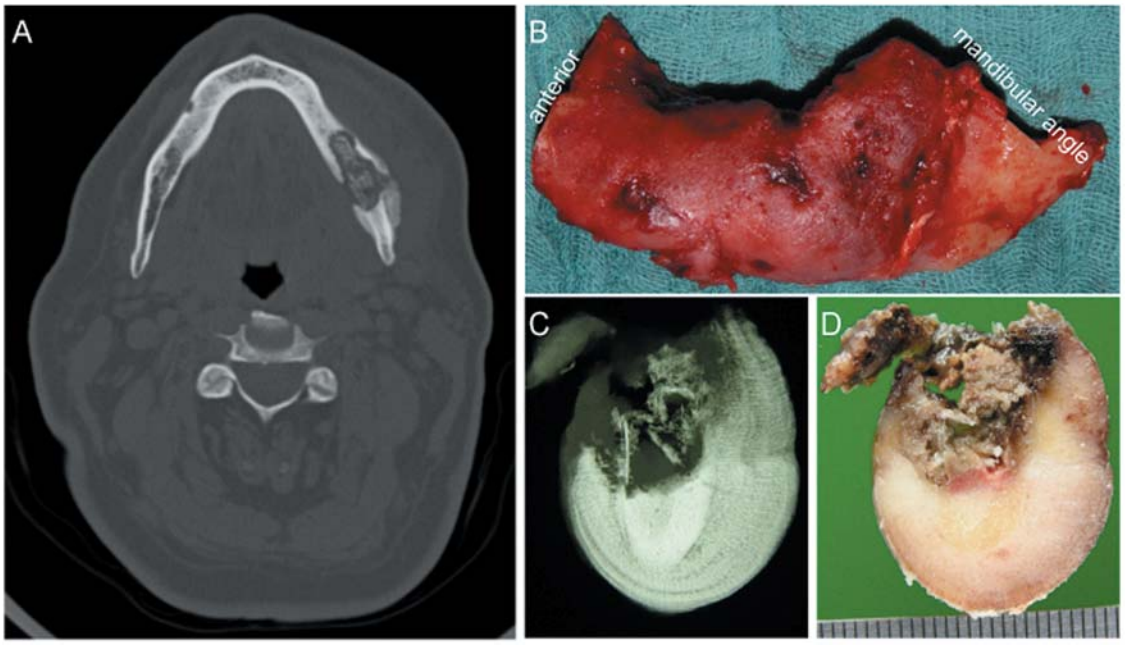

Figure 1. *BRONJ, gross findings. (A) Clinical CT showed an osteolytic jaw bone lesion with central sequester. (B) The surface of the jaw bone was covered with new periosteal bone formation. (C) Slab radiograph revealed a central sequester, and the remainings of cortical bone with concentric lamellated new periosteal bone formations. (D) Macroscopically, brownish necrotic tissues were apparent.*

seems analogous to other inflammatory oral lesions (21, 24) and should, thus, not be considered a sign of malignancy.

Based on our results it can be stated that histopathological diagnosis of BRONJ is unproblematic as long as surgical pathologists obtain relevant clinical data and larger excision specimens for their analysis. However, it can be quite problematic to confirm the clinical diagnosis in cases with only small or fragmented biopsy specimens taken from the border of the lesion, which might contain only viable inflamed granulation tissue without bone sequester. Altogether, the current roles of the surgical pathologist in diagnosing BRONJ are firstly to confirm the presence of BRONJ, and secondly to exclude a malignancy. Even though some authors have concluded that BRONJ is quite similar to jaw osteonecrosis associated with radiotherapy treatment (34), other researchers observed some substantial differences between the two lesions; of particular interest were the vessel obliteration (12) and patchy pattern of osteonecrosis (4) in BRONJ. Although malignant tumors have only rarely been observed in association with BRONJ (43-46), malignancy must also be excluded by histopathological analysis. In our study cohort, we did not observe malignant tumor infiltrates in BRONJ lesions.

With further development of surgical methods, one possible future role of the surgical pathologist might be validation of minimal but efficient curative surgery performed in BRONJ cases. Indeed, intraoperative visualization of viable versus necrotic bone areas by means of fluorescence-guided bone resection following doxycycline labeling of newly-formed bone has recently been shown to be useful in detecting the borders of BRONJ lesions (47). This and/or other novel surgical techniques might help define the best possible time for and extent of surgery in BRONJ cases in the future.

Conclusion

We demonstrated podoplanin expression in non-neoplastic pseudoepitheliomatous hyperplasia associated with BRONJ lesions. We discussed the current and possible future roles of surgical pathologists in diagnosing morphological changes associated with the development and therapy of BRONJ lesions.

Conflicts of Interest

We declare that we have no conflicts of interest (either financial or personal) in regard to this study. This work has not been published or presented (oral, print or online) elsewhere in whole, nor in part.

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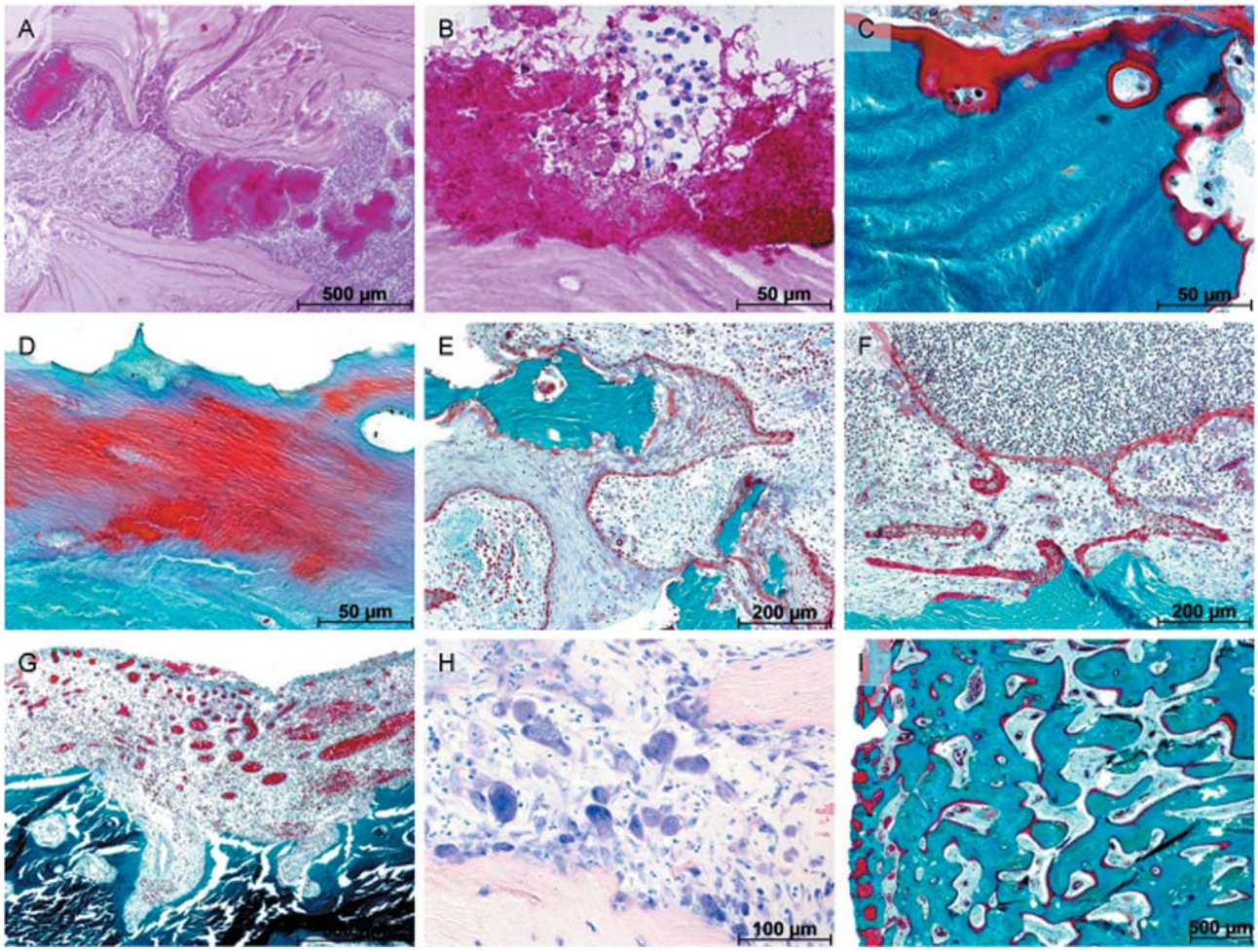

Figure 2. *BRONJ, histopathological findings. (A) In between the osteosclerotic bone trabeculae, granulation tissue and bacterial colonies were apparent under low-power microscopy (periodic acid-Schiff; original magnification: ×50). (B) On the surface necrotic bone tissue, Actinomyces bacteria and neutrophils were seen under higher-power microscopy (periodic acid-Schiff, ×400). Areas of de-mineralized bone matrix were seen both (C) superficially and (D) within the deeper bone regions (C/D: Goldner trichrome, ×400). (E) Some BRONJ lesions showed fragments of necrotic bone sequester embedded in inflamed granulation tissue and pseudoepitheliomatous hyperplasia. (F) Similarly, pseudoepitheliomatous hyperplasia covering the bottom of BRONJ lesions was in some cases covered by purulent exudate. (G) Viable bone in several BRONJ lesions with desquamated bone sequester were covered by granulation tissue showing hyperemia and superficial seam of pseudoepitheliomatous hyperplasia (E/F/G: Goldner trichrome, ×100). (H) Deeper viable bone tissue showed often numerous osteoclasts within the proliferated granulation tissue (Giemsa, ×200). (I) The majority of the resection specimens showed distinct new periosteal bone formation (Goldner trichrome, ×50).*

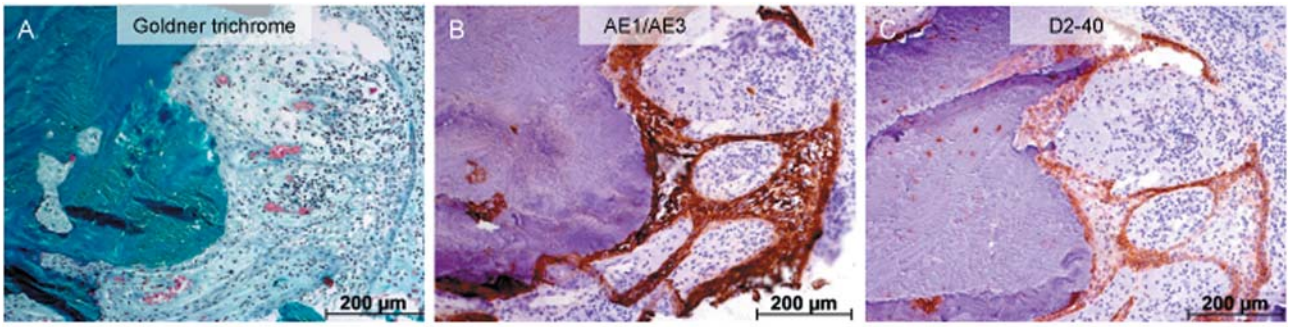

Figure 3. Pseudoepitheliomatous hyperplasia. (A) Pseudoepitheliomatous hyperplasia was apparent within the inflamed granulation tissue (Goldner trichrome, ×100). (B) Epithelial nature of the proliferated epithelium was highlighted using immunohistochemistry (AE1/AE3, ×100). (C) Podoplanin expression was seen in basal and parabasal layers (D2-40, ×100).

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