Original Article
STAT3 is a key pathway in primary intraosseous squamous cell carcinoma arising from an odontogenic keratocyst

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Abstract: Purpose: Primary intraosseous squamous cell carcinoma (PIOSCC) arising within an odontogenic keratocyst (OKC) is rare malignancy, entailing a poor prognosis for delayed diagnosis. The number of reports concerning this entity is extremely small. The aim of this study is to present the clinical and pathologic characteristics of PIOSCC and investigate its pathogenesis. Materials and methods: This study describes three patients with mandibular PIOSCC derived from an OKC over a seven-year period, each of which suffering from decompression in mandible. Important diagnostic criteria included the absence of overlying oral mucosal ulceration, the absence of a distant potential primary tumor, and the presence of a completely intraosseus lesion. The malignant transformation of OKC to PIOSCC was confirmed by pathologic evaluation of surgical resection specimens. Immunohistochemistry (IHC) was used to evaluating expression of Ki-67, p65, EGFR, phospho-AKT, and STAT3 in each of the three tumors and adjacent cyst walls. Results: Analysis by IHC indicated that Ki67, P65, EGFR and STAT3 were substantially elevated in PIOSCC. There was an obvious positive correlation between Ki67, P65, EGFR and STAT3 expression in adjacent benign epithelium. Each tumor exhibited long-standing chronic inflammation in the benign odontogenic cyst, suggesting that a sustained immune response may be partially responsible for malignant transformation of the benign cystic lining cells. Conclusions: These findings indicate that inflammation may be the principal mediator in PIOSCC ex-OKC, and the STAT3 signaling pathway is an important contributor to this process. Combined detection of Ki67, P65, and EGFR in the lesional epithelium can support the diagnosis of PIOSCC.

Keywords: Primary intraosseous squamous cell carcinoma, odontogenic keratocyst, decompression, inflammation, STAT3

Introduction

Odontogenic keratocyst (OKC) has been traditionally termed as odontogenic cysts previously. Currently, according to the most recent WHO classification, this entity is considered a distinct odontogenic tumor [1]. Previous studies have focused on the unique characteristics of this entity, which putatively originates from the dental lamina remnants of the jaw. Different methods of treatment for OKC include enucleation and decompression, the latter of which is a reliable method for preserving important facial structures and has been considered acceptable due to a low risk of recurrence. Post-operative malignant transformation of OKCs into squamous cell carcinoma (SCC) is a very rare occurrence which occurs only in the jaw [2]. A definitive diagnosis of PIOSCC is often troublesome for the pathologist, mainly due to difficulty in distinguishing this entity from gingival carcinomas and distant metastases of unknown primaries [3]. Additionally, the etiology of this transformation is still unclear. In the following article, we present three clinical cases of OKC located in the mandibular (**Table 1**), which had been treated by decompression and subsequently transformed into PIOSCC. The purpose of this article is to discuss the clinical features that may aid in the early diagnosis of this lesion. Additionally, the potential of long-standing chronic inflammation in post-operative

Table 1. Data of 3 patients with PIOSCC

Case Age	Sex	Site	Signs and Symptoms distant metastases	Lymph node and distant metastases	Treatment	Follow-up
57	F	Posterior mandible	Paresthesia, pain	Neg	hemimandibulectomy SOND + radiotherapy	no evidence of disease 7 months
47	M	Anterior mandible	Swelling	Neg	hemimandibulectomy radiotherapy	no evidence of disease 3 months
72	F	Posterior mandible	Asymptomatic	Neg	hemimandibulectomy SOND	no evidence of disease 8 months

OKC is discussed as a potential etiology factor in the pathogenesis of this malignancy.

Materials and methods

The study design for this research was approved by an institutional review board of the Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine China. Specifically, the human subject protocol was approved by the Committee on Clinical Investigation. The clinical diagnosis was confirmed in the Department of Oral-Maxilla Head and Neck Surgery at Ninth People's Hospital Shanghai, and informed consent was collected from all patients for this investigation, in accordance with the Declaration of Helsinki.

Classical case presentation

A 57-year-old male patient presented with complaints of mandibular swelling in the left molar region. Radiologic imaging demonstrated a well-defined unilocular radiolucency surrounding an impacted mandibular left third molar (**Figure 1A**), and computerized tomography (CT) imaging suggested expansion and thinning of both the buccal and lingual cortical plates (**Figure 1B**, **1C**). Subsequently, the patient underwent decompression treatment under general anesthesia after the pathologic diagnosis of OKC was made (**Figure 1D**). Afterward, the patient was instructed to irrigate twice daily to prevent food accumulation and closure of the fistula. Post-operative follow-up was performed every three months, and plain film radiographs of the patient was obtained (**Figure 2A-F**). The formation of new bone trabecula was observed overtime in the panoramic films, with gradual reduction of the jaw cavity. Twenty-one months post-operatively, the patient presented with recurrent swelling which became painful one month later. The patient also experienced numbness of the left lower lip, and extraoral examination revealed a firm, nontender swelling in the left mandibular body region. A chronic discharging sinus was observed in the left inferior border of mandible skin. According to the patient, this sinus tract had been present for at least 3 months with recurrent discharge of pustular fluid. Intraoral examination revealed that the overlying alveolar mucosa was intact without evidence of ulceration. A firm, nontender swelling was present in the left mandibular molar region with buccal and lingual cortical expansion. There was no enlargement of the cervical lymph nodes, and an orthopantomogram demonstrated the disappearance of newly-formed bone trabecula as well as a unilocular radiolucency with ill-defined margins extending from the distal root of the lower left first molar to the sigmoid notch of the ascending ramus of the left mandible (**Figure 3A**, **3B**). CT imaging revealed a large oval mass at the left angle of the mandible with extensive bony destruction of the lingual and buccal cortex (**Figure 3C**, **3D**), and an incisional biopsy was performed. Pathological examination revealed non-keratinized stratified squamous epithelium with severe dysplastic features. Sheets and islands of anaplastic squamous cells with marked nuclear pleomorphism, hyperchromatic nuclei, keratin pearl formation and individual cell keratinization was also observed, resembling well-differentiated SCC (**Figure 3E**, **3F**). The adjacent connective tissue demonstrated moderate chronic inflammatory infiltrate, and the diagnosis of PIOSCC arising from an odontogenic keratocyst was made. The patient underwent resection of the mandible with 1.5-cm healthy bone margins with supraomohyoid neck dissection, and the patient was disease-free 1 year status-post resection.

Antibodies and reagents

The antibodies used for immunohistochemistry and analysis included anti-Ki67 (ab155-80, Abcam), anti-P65 (ab16502, Abcam), anti-EGFR (Santa Cruz, SC-16803), STAT3 (ab30647, Abcam).
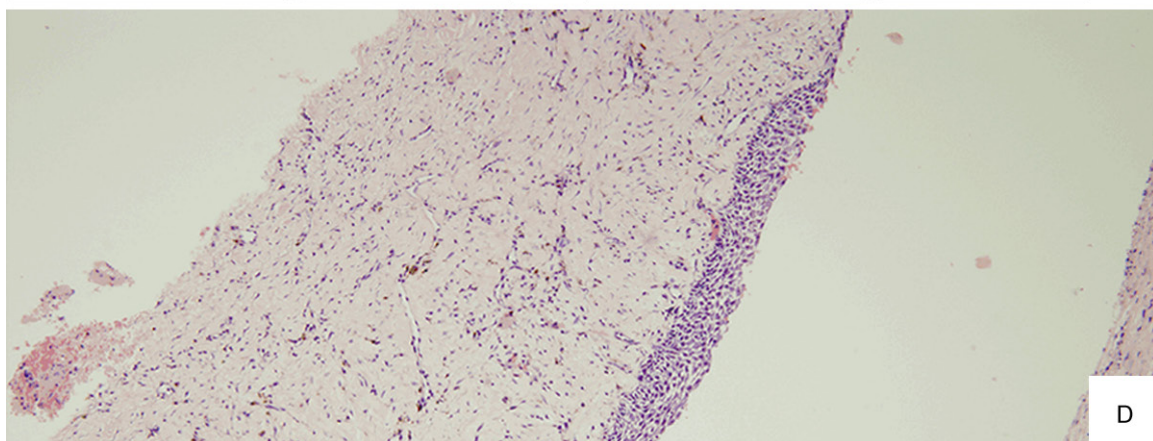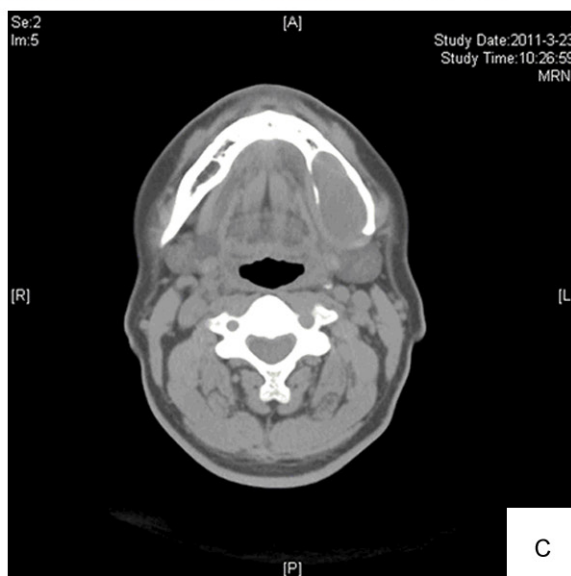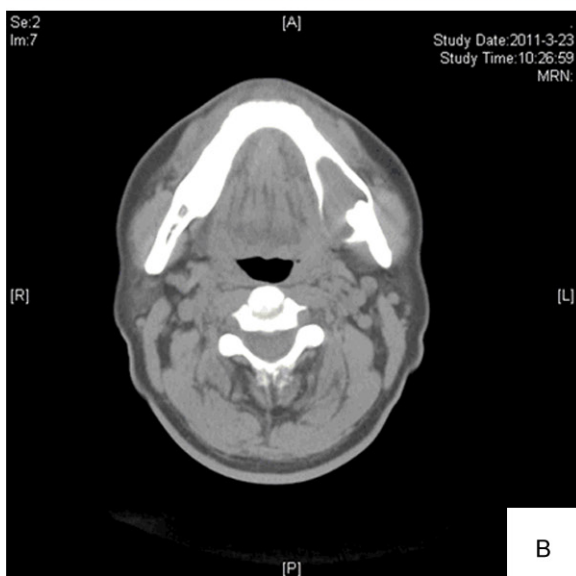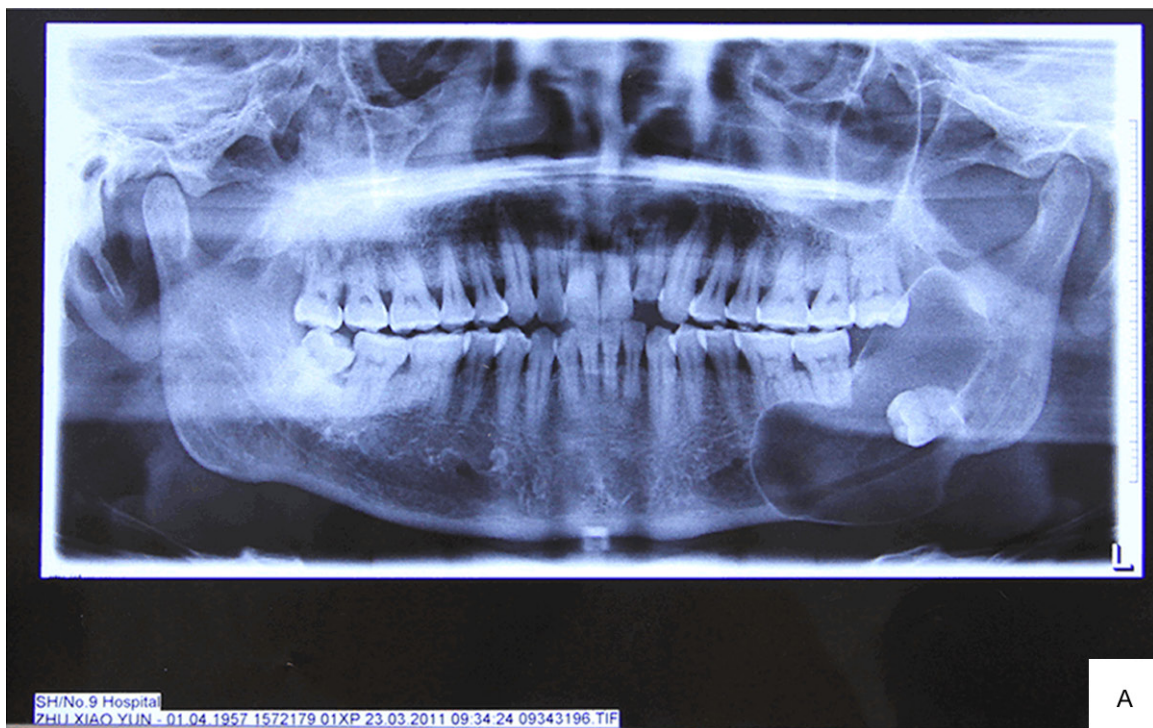
Figure 1. A. Panoramic radiograph of PIOSCC showing a well-defined unilocular radiolucency in the postmolar-molar area of the left posterior mandible; B, C. CT image of PIOSCC showing a well-defined unilocular osteolytic lesion in the posterior mandible; D. Histologic features of Odontogenic Keratocyst. (H&E, ×400).
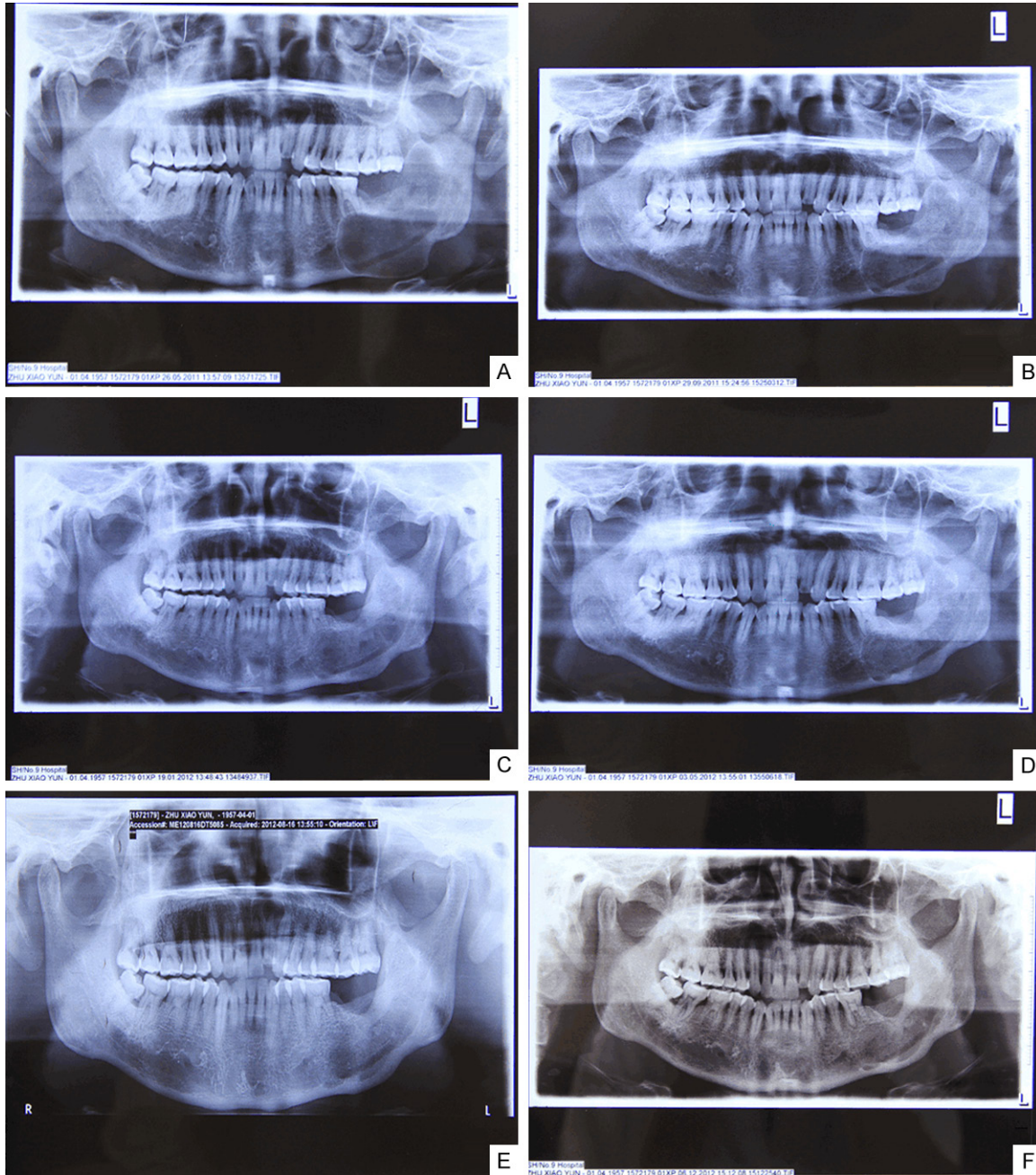

Figure 2. Panoramic film of postoperation follow up every three months.

Immunohistochemistry

Tissue specimens were fixed in formalin and embedded in paraffin. Serial sections, 5 µm in thickness, were cut from tissue blocks. After rehydration and de-waxing, the sections were subjected to an antigen retrieval protocal including incubation in citric acid, incubated with 3% H_2O_2 for 10 min and blocked with 5% BSA for 20 min. Specimens were then incubated with the primary antibody (anti-Ki67 1:500, anti-P65 1:200, anti-EGFR 1:300, anti-STAT3
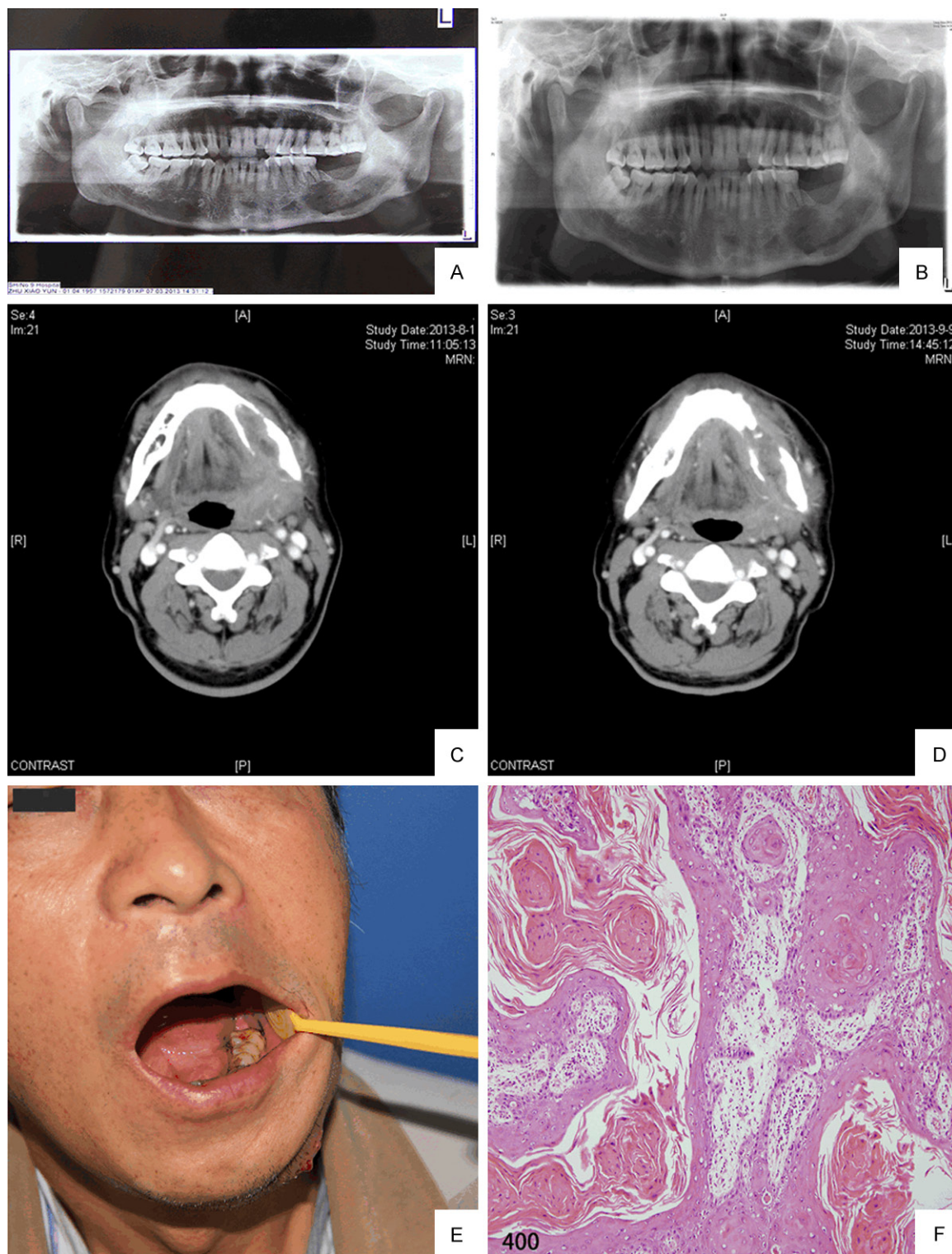
Figure 3. A, B. Orthopantomograph showing gross destruction of left body and ramus of the mandible; C, D. CT image revealing a large bony destruction of the lingual and buccal cortex from the ascending ramus to body of the mandible; E. There was no lesion to be found in the oral; F. Pathological examinationshowing infiltrative atypical squamous cell nests (H&E, ×400).

1:100) for 24 h at 4°C. Secondary antibody was added (HRP) and incubated for 1 h, followed by the addition of 100 µL of diaminobenzidine (DAB). Dehydration, transparence, mounting
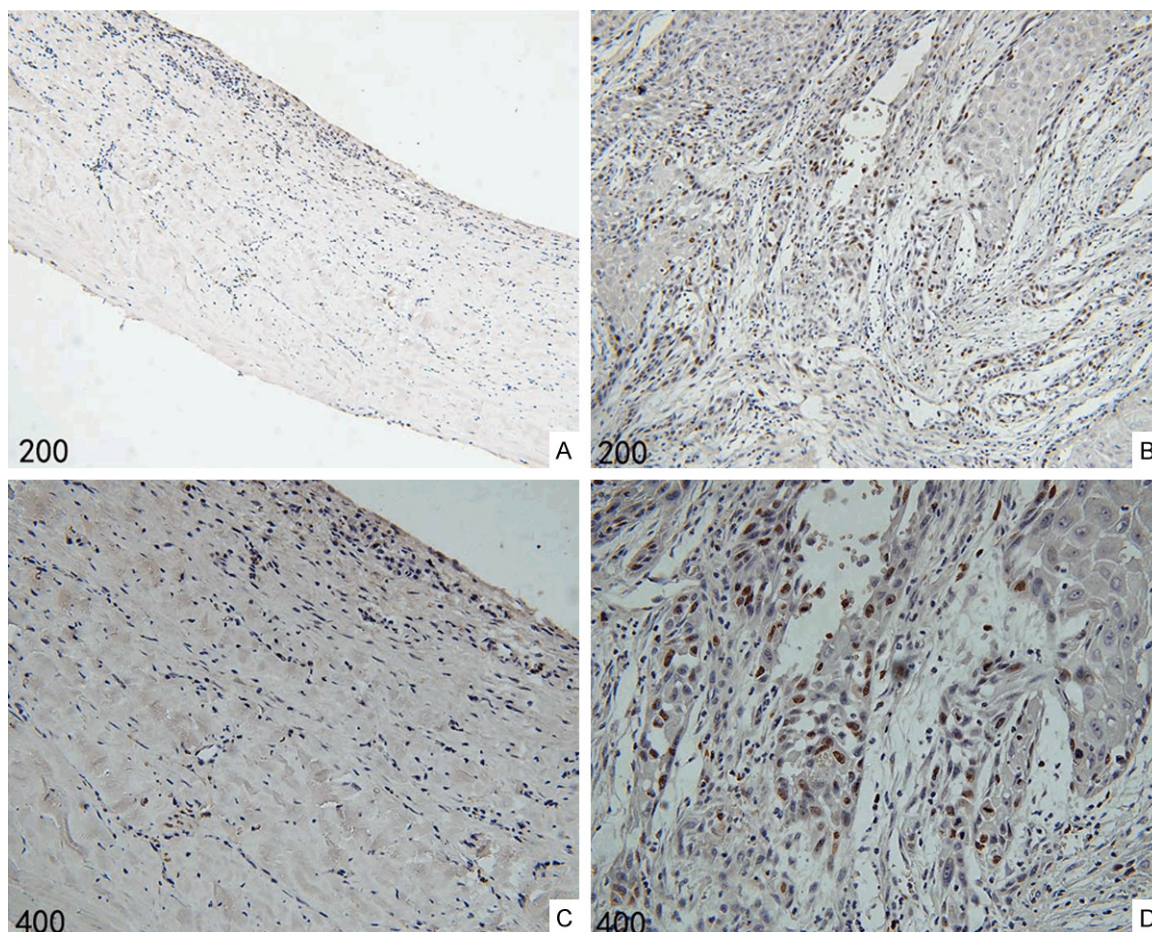
Figure 4. Ki-67 expression in OKC and PIOSCC. A, C. Ki-67 expressions in the OKC. B, D. Ki-67 expressions in the PIOSCC.

and histological images were obtained using an Olympus light microscope (BX50).

Results

Ki67 immunohistochemistry statistical analysis

Ki67 was positive in cytotrophoblasts and squamous epithelium cells. Compared to the OKC, expression of Ki67 was increased significantly. Ki67 staining density of squamous epithelium cells was statistically significantly lower in the OKC compared to PIOSCC (**Figure 4A-D**).

Expression of p65

In benign OKC, p65 exhibited weak expression within the epithelial nuclei. In the associated inflammatory cells there was strong p65 expression in the cellular membranes. It was observed that expression of p65 was progressively increased as lesions transitioned from

intraepithelial to invasive squamous components. In malignant PIOSCC, p65 was expressed in the epithelial nuclei with focal positive expression observed within the cytoplasm (**Figure 5A-D**).

Expression of EGFR and STAT3

In both OKC and PIOSCC specimens, EGFR positive cells were confined to the lower portion of mid epithelial zone. Immunohistochemical staining confirmed the EGFR and STAT3 were strongly positive in PIOSCC and negative in OKC. Immunohistochemical analysis showed that EGFR was strongly positive expressed at cytomembrane (**Figures 6A-D**, **7A-D**).

Discussion

PIOSCC is an odontogenic epithelial malignancy arising from residual odontogenic epithelium within the bone. The diagnostic criteria formally suggested for PIOSCC include the absence of
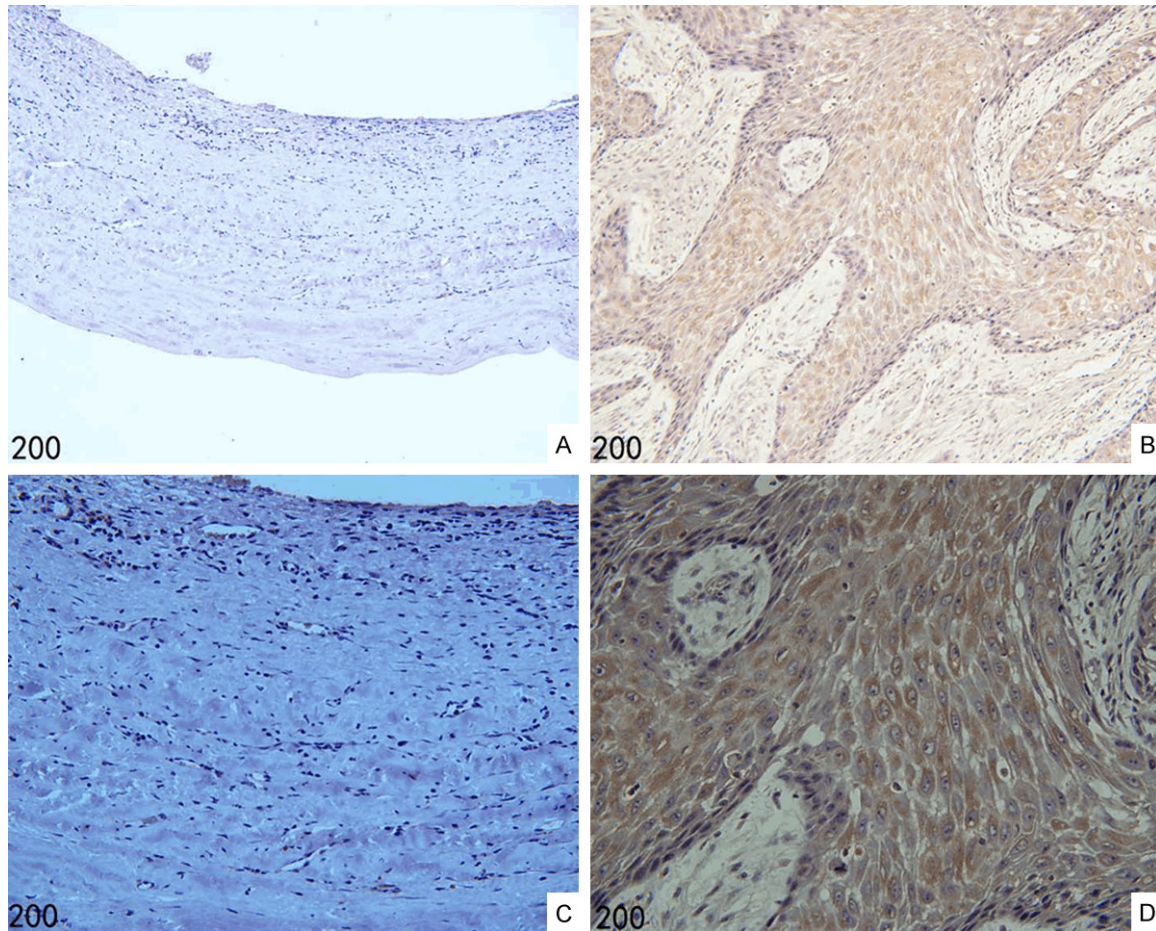
Figure 5. P65 expression in OKC and PIOSCC. A, C. P65 expressions in the OKC. B, D. P65 expressions in the PIOSCC.

a direct connection with the alveolar mucosa, histologic evidence of squamous cell carcinoma without other odontogenic tumor cells and in the absence of another primary tumor, and an ideal follow-up period of more than 6 months [4]. The ratio of this malignancy in males to females is 2 to 1, with an estimated mean age of 50 years [5]. The PIOSCC ex-OKC is a rare malignant odontogenic tumor, with only 16 cases previously reported in the literature [6].

The most common symptoms of patients with PIOSCC are sensory disturbances such as numbness and paresthesia. Swelling and pain can also occur. Perforation of the buccal of mandible was seen in one patient in our study. Metastasis to cervical lymph nodes is rare, and no cervical lymph nodes metastasis was seen in our study. It has been previously reported that the presence of lymph node metastases is a significant prognostic factor for recurrence. As cited in the literature, approximately 50% of

cases of PIOSCC show metastasis to the cervical lymph nodes, and, in these cases, adjunctive radiation therapy may result in improved outcomes when combined with surgical excision [7], with a five-year survival rate of 30% to 40% [8]. Prognosis is directly related to complete and adequate resection, which requires detection early before the presence of metastasis to cervical lymph nodes or the extensive involvement of adjacent tissues.

The presence of a lesion with indistinct margins lacking a sclerotic outline is an important radiographic feature of PIOSCC. Panoramic radiography is of limited value in evaluating the margin, extension and invasion of these lesions [9]. CT imaging better allows visualization of these aggressive and is certainly indicated in cases of potentially malignant mandibular tumors [10]. The histopathologic features of PIOSCC is consistent with a diagnosis of squamous cell carcinoma. For the diagnosis, a cys-
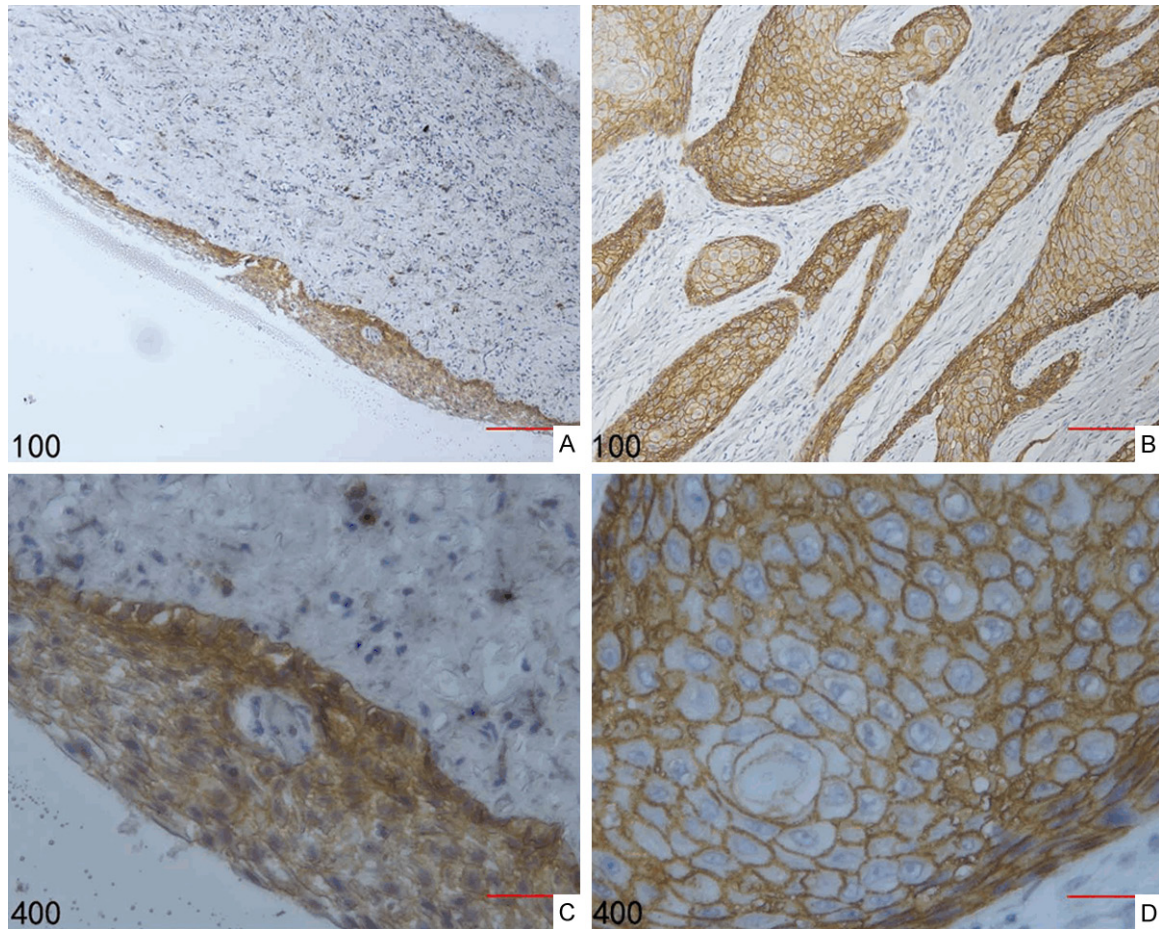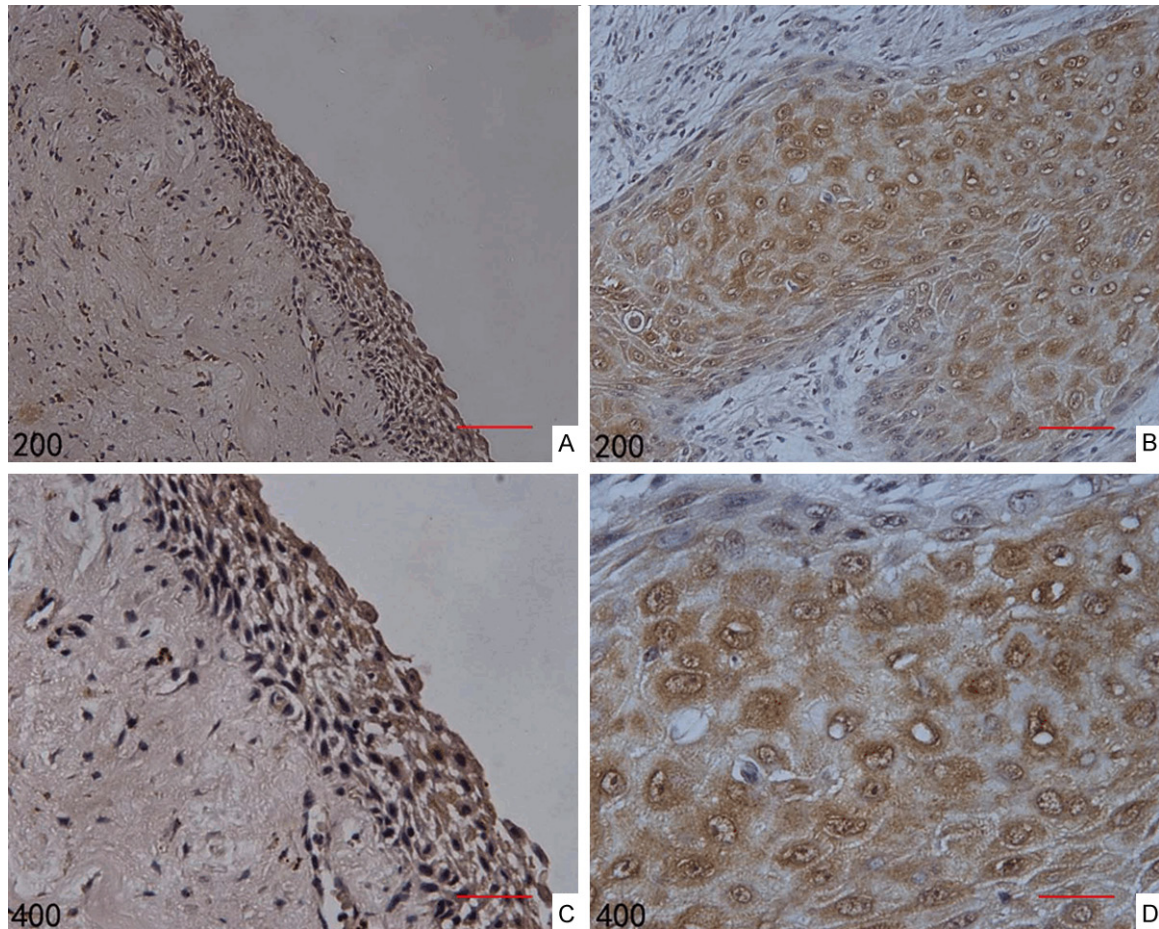
Figure 6. EGFR expression in OKC and PIOSCC. A, C. EGFR expressions in the OKC. B, D. EGFR expressions in the PIOSCC.

tic component is a prerequisite for the diagnosis [11]. In our study, all cases of PIOSCC exhibited features of a well-differentiated keratinizing carcinoma which developed from a previously treated odontogenic keratocyst. PIOSCC must be considered in the differential diagnosis of malignant tumors of odontogenic epithelium, and squamous cell carcinoma of mucosal origin should be definitively ruled out before a diagnosis can be made [12].

The pathogenesis of PIOSCC ex-OKC is unclear at the present time. Based on the notion that SCC of odontogenic origin arises not only in the bone marrow, but also in the periodontal and the subepithelial soft tissue spaces, some authors have suggested that the term "odontogenic squamous cell carcinoma" replace PIOSCC [13]. OKC linings seem to have higher mitotic activity than those of other odontogenic cysts, with a greater (albeit still rare)

potential to evolve into SCC [14]. A potential association between inflammation and malignancy has been postulated in a wide variety of cancers [15]; however, a precise biologic mechanism linking inflammation and cancer development is still obscure. The most common factor is thought to be a long-standing chronic inflammatory stimulus with a predisposing genetic cofactor that facilitates neoplastic transformation [16]. It is thought that chronic inflammation promotes apoptosis of the involved cells, leading to proliferation of active tumor stem cells, which become subjected to the effects of cumulative mutations [17]. Several proteins are involved in the early steps of OKC carcinogenesis, such as NF-κB, lipoxins, and arginase [18]. These molecules are involved in continuous cross-talk between the epithelial and the stromal components. Recent evidence has suggested that the stroma plays an important role in influencing carci-

Figure 7. Expression of STAT3 in OKC and PIOSCC. A, C. STAT3 expressions in the OKC. B, D. STAT3 expressions in the PIOSCC.

nogenesis [19]. Carcinogens caused by chronic inflammation in the cyst wall may originate from the oral bacterial environment. Lymphocytes, neutrophils and macrophages are among the immune cells mostly involved in these processes, and proteins, such as resolvins and cyclooxygenases, are crucial in these inflammatory pathways [20]. Moreover, the activation of these pathways establishes an oxidative environment which causes DNA damage and leads to a shift from an aerobic to an anaerobic metabolism, thus inducing proliferation, apoptosis and autophogy, ultimately causing final failure to control normal cell repair and renewal [21]. In our study, cell cycle proteins Ki67, p65, EGFR and STAT3 were expressed positively. We propose that STAT3 are a core signal transduction pathway in the pathogenesis of PIOSCC ex-OKC. However, the picture of the early events in OKC carcinogenesis is still incomplete, and future studies are needed in order to draw more definitive conclusions.

Conclusion

Long-standing chronic inflammation may play an important role in the pathogenesis of PIOSCC ex-OKC; therefore, it is necessary to irrigate the fistula after decompression to prevent food accumulation which can lead to inflammation. The signs of chronic inflammation in OKC should be taken into consideration when evaluating patients for recurrence, and such lesions should be diagnosed treated promptly.

Disclosure of conflict of interest

None.

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