Transformation of an odontogenic keratocyst into a solid variant of odontogenic keratocyst/keratoameloblastoma during long-term follow-up: A case report

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Abstract. Keratoameloblastoma (KA) and solid variant of odontogenic keratocyst (SOKC) are rare odontogenic lesions, and their relationship and differences are unclear. The present study described a case that started as an odontogenic keratocyst (OKC) and transformed to SOKC/KA upon recurrence. Briefly, a 26-year-old man presented with swelling in the right cheek and was referred to the Department of Oral and Maxillofacial surgery, Hiroshima University Hospital (Hiroshima, Japan). At the initial visit, unicystic bone permeation was observed extending from the right canine to the molar, maxillary sinus and nasal cavity. After the biopsy, the patient underwent excisional surgery and was diagnosed with OKC. Thereafter, the lesion recurred six times over a period of 13 years and showed different histopathological features from those of the primary lesion, all consisting of numerous cysts with keratinization, which were diagnosed as SOKC/KA. The Ki-67 positivity rate was ~10%, which was higher than that of the primary lesion, but there was no atypia. Genetic analysis of the recurrent lesion revealed mutations in adenomatous polyposis coli and Kirsten rat sarcoma viral oncogene homolog. This case originated from OKC, and the morphological features of OKC and KA were mixed upon recurrence, supporting the commonality and association between the two. However, multiple mutations different from those of OKC and ameloblastoma were detected, suggesting an association of SOKC/KA with increased proliferative activity and a high recurrence rate.

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Abbreviations: KA, keratoameloblastoma; OKC, odontogenic keratocyst; SOKC, solid variant of OKC; AB, ameloblastoma; APC, adenomatous polyposis coli; KRAS, Kirsten rat sarcoma viral oncogene homolog

Key words: SOKC, KA, OCK, *APC*, *KRAS*

Introduction

An odontogenic keratocyst (OKC) is a distinctive cyst lined with a thin and flat parakeratotic squamous epithelium composed of a palisaded basal layer and a corrugated surface. Although the most recent World Health Organization classification (2022) of odontogenic lesions defines OKC as a developmental cyst, it differs from other odontogenic cysts in that it displays potentially aggressive behavior due to the high proliferative activity of the epithelium, and high rates of recurrence and genetic alterations (1). In addition, the presence of a solid variant of OKC (SOKC), which is composed of numerous small, keratinized cysts and epithelial islands characterized by palisaded basal cells with hyperchromatic nuclei in the fibrous connective tissue, supports its benign neoplastic nature (2,3). The clinical and histopathological features of SOKC may overlap with those of keratoameloblastoma (KA), a rare variant of ameloblastoma (AB); however, focal stellate reticulum-like areas, subnuclear vacuolization and lamellated-type central keratinization have been reported to be key in the diagnosis of KA (2). Some researchers have considered SOKC and KA as histogenetically related entities that represent a continuous spectrum of a single tumor type (2,3). Zhang *et al* (1) reported that SOKC and KA are difficult to distinguish because both are rare lesions with similar clinical, histopathological and biological features (1). To date, to the best of our knowledge, two cases of SOKC and six cases of KA in the maxilla have been reported (1). The concept of SOKC/KA has been proposed based on the clinicopathological similarities between these two lesions. The present study describes a case of SOKC/KA in which primary unicystic OKC recurred as multiple keratotic islands and microcysts; therefore, we aimed to characterize the histogenetics of SOKC/KA by performing a genetic analysis and a review of the literature.

Case report

Patient history. A 26-year-old man who presented with swelling and pain in the right buccal region in February 2009 was referred to the Department Of Oral and Maxillofacial Surgery, Hiroshima University Hospital (Hiroshima, Japan). Radiographs showed permeation of the root apex of a right maxillary canine. The patient had no family history of any other diseases, and was a nonsmoker and nondrinker. Blood tests revealed normal complete blood count liver function, as determined by detecting alanine transaminase and aspartate transaminase, alkaline phosphatase and γ-glutamyl transferase, and renal function, as determined by detecting blood urea nitrogen, creatinine and estimated glomerular filtration rate. At the initial visit, no buccal sensory abnormalities were observed, but bony swelling occurred from the right maxillary canine to the molar region, causing root avulsion between the canine and the first premolar (Fig. 1A and B). CT imaging before the treatment showed that the right nasolacrimal duct was obstructed by the lesion, and radiographs showed a lesion extending from the right nasal cavity to the right maxillary sinus, with thinning and bulging of the buccal cortical bone and posterior wall of the right maxillary sinus, resorption of the lateral wall of the right nasal cavity, and thinning of the suborbital wall (Fig. 1C and D). Biopsy findings indicated an OKC, and extraction of the right maxillary cyst and radical maxillary sinus surgery were performed in March 2009. The tumor was encased in a capsule and was removed in one piece, and the superficial maxillary bone was removed with a round bur. The cyst was macroscopically found to have a single cavity lined with a thin wall. Microscopically, despite a careful search, the proliferation of microcysts or solid islands with keratinization within the cyst wall were not observed (Fig. 1E and F). At 2 and 4 years of follow-up, the lesion recurred, and thus, the lesion was removed twice more. A total of 7 years after the initial visit, the recurrence was found in the right maxillary canine, and the first and second premolar areas. Cone-beam CT imaging showed that a lesion with well-defined but slightly uneven margins was observed, some of which were in close proximity to the nasal floor on the side of the maxillary sinus crest from the right canine tooth (Fig. 2C-E). The tumor was removed, and the first premolar was extracted. After 2 years of close follow-up (9 years after the initial visit), the disease recurred in the same area. The recurrent tumor and surrounding bone were then removed (Fig. 2A-E) and was histopathologically diagnosed as SOKC/KA (Fig. 2F and G). A total of 10 years after the initial visit, the tumor recurred in the second premolar area; therefore, the patient underwent tumor removal and second premolar extraction. Treatment progress is shown in Table SI. At present (14 years and 4 months after the initial visit), no further recurrence has been observed, but the patient continues to undergo strict periodic follow-ups.

Immunohistochemistry (IHC). Immunohistochemical staining of sections from the formalin-fixed paraffin-embedded tissue samples was performed using Ventana BenchMark XT slide stainer (Ventana Medical Systems, Inc.). IHC was performed to detect p53 (DO7; cat. no. 790-2912; Roche Diagnostics K.K; prediluted), Ki-67 (30-9; cat. no. 790-4286; Roche Diagnostics K.K; prediluted), BRAF (VE1; cat. no. 790-5095; Roche Diagnostics K.K; prediluted), calretinin (SP65; cat. no. 790-4467; Roche Diagnostics K.K; prediluted), β-catenin (β-catenin-1; cat. no. GA70261-2; Agilent Technologies Japan, Ltd.; prediluted) and CD56 (MRQ-42; cat. no. 418191; Nichirei Biosciences Inc.; prediluted) expression in primary OKC and recurrent (10 years after the initial diagnosis) SOKC/KA tissue specimens. In addition, phosphorylated (p)-S6 ribosomal protein (S6) (Ser235/236; cat. no. 2211S; Cell Signaling Technology, Inc.; 1:500) and p-ERK1/2 (Thr202/Tyr204; cat. no. 20G11; 4376; Cell Signaling Technology, Inc.; 1:500) were investigated in the same recurrent lesions. IHC was performed on 5-μm sections of tissues fixed for 24 h at room temperature in 10% neutral buffered formalin solution and embedded in paraffin. After microwave-based epitope retrieval for 5 min (two times), sections were incubated for 20 min at room temperature in 10 mM citrate buffer (pH 6.0). Next, endogenous peroxidase was blocked with 3% hydrogen peroxide at room temperature for 15 min. Incubation with 2.5% BSA (MilliporeSigma) in PBS for 10 min at room temperature was used to block non-specific reactions. The sections were exposed for 1 h at room temperature with each primary antibody. Incubation with secondary antibodies (horseradish peroxidase-conjugated goat anti-rabbit antibody; cat. no. ab6721; 1:1,000; or horseradish peroxidase-conjugated goat anti-mouse antibody; cat. no. ab6789; 1:500; both Abcam]) was conducted for 1 h at room temperature and detection was performed using the Ventana UltraView Universal DAB Detection kit (Ventana Medical Systems, Inc.) according to the manufacturer's instructions. The sections were observed under a light microscope (Nikon Eclipse E800 microscope; Nikon Corporation).

DNA isolation and gene panel sequencing analysis. Extraction and purification of genomic DNA from 5-μm tissues of the recurrent lesions diagnosed as SOKC/KA (13 years after the initial diagnosis) was performed by an outside contractor (Macrogen Inc.). Initially, the tissues were fixed in 10% formalin at room temperature for 24 h and embedded in paraffin. Analysis of the genes listed in Table SII [single nucleotide variants (SNVs)/insertion-deletions (InDels) (170 genes) and fusions (25 genes)] was performed using next-generation sequencing with Axen™ Cancer Panel 2 (Macrogen Inc.). Written informed consent was obtained from the patient, including consent for participation and publication of the findings. This work was approved by the Ethics Committee of Hiroshima University (approval number: hi-72; Hiroshima, Japan).

Primary and recurrent lesions exhibit different histopathological features. The primary lesion was a single cyst lined with a thin, stratified squamous epithelium of uniform thickness with no rete ridges. It was diagnosed as OKC based on the characteristic parakeratinized luminal surface with a focal corrugated appearance and the palisaded basal cell layer, although orthokeratinized areas were present in H&E (Fig. 1E and F). Sections were prepared to a thickness of 5-μm and stained with H&E using the Tissue-Tek® Prisma™ Plus automated slide stainer (Sakura Finete Japan Co., Ltd.) and observed under the light microscope. No daughter cysts or epithelial islands were observed within the cyst walls. The

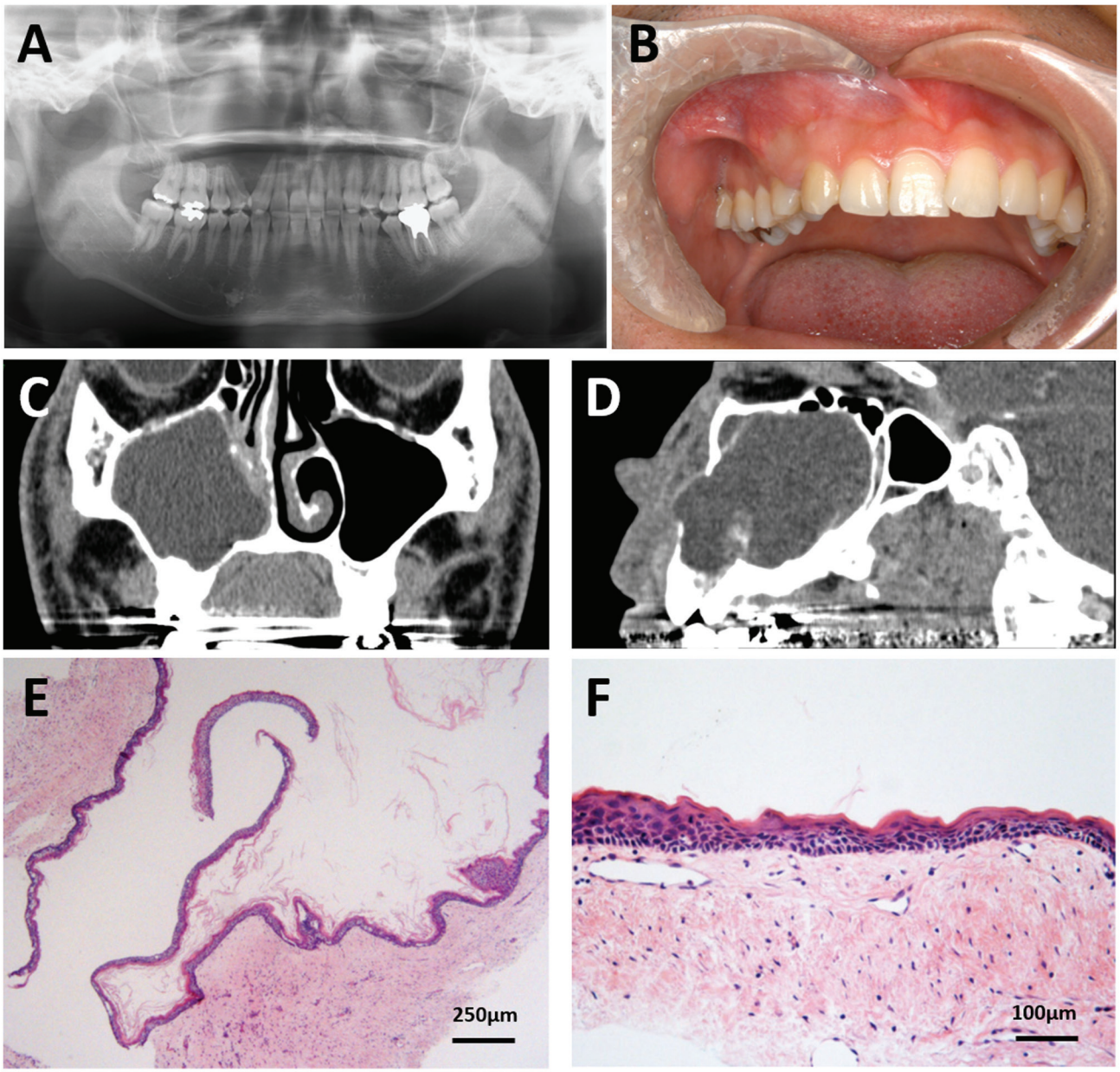

Figure 1. Clinical conditions at initial examination. (A) Panoramic radiograph at initial examination. (B) Intraoral photograph before treatment. (C) Coronal plane and (D) sagittal plane of CT imaging before treatment. The lesion was located from the right maxillary sinus to the right nasal cavity. The buccal cortical bone was thinning and bulging. The posterior wall of the right maxillary sinus was also thinned and slightly distended, the bone wall of the lateral wall of the right nasal cavity was resorbed, and the suborbital wall was thinning. (E and F) Histopathological analysis of primary odontogenic keratocyst. Hematoxylin and eosin staining; (E) magnification, x40 and (F) magnification, x100. A single cyst lined by thin stratified squamous epithelium of uniform thickness with no rete ridges was detected. The parakeratinized luminal surface with focal corrugated appearance and the palisaded basal cell layer was characteristic of OKC.

six lesions that recurred over 13 years exhibited histopathological features different from those of the primary lesion. They consisted of a number of small cysts and solid epithelial nests with parakeratinization and central lamellated keratin accumulation. Focal areas of loosely arranged polygonal epithelial cells resembling the stellate reticulum of the enamel organ and reverse nuclear polarity of the basal cells were also observed. Moreover, the recurrent lesion consisted of OKC, AB with prominent central keratinization, and epithelium with features of both. Although the staining for p53 in recurrent lesions was slightly higher than that in primary lesion and the Ki-67 labeling index of the epithelial cells in the recurrent lesions (~10%) was higher than that in the primary lesion (~4%), cellular and nuclear pleomorphisms were not prominent in any lesion and malignant transformation was ruled out due to the absence of cellular atypia (Fig. 3). The recurrent lesions were diagnosed as SOKC/KA. IHC results showed that calretinin, CD56 and BRAFV600E were not expressed in either the primary or recurrent lesions (data not shown). These findings indicated that this lesion is different from typical AB or OKC.

Cancer panel sequencing analysis shows mutations in adenomatous polyposis coli (APC) and Kirsten rat sarcoma viral oncogene homolog (KRAS). SNVs/InDels (170 genes) and fusions (25 genes) were analyzed in the

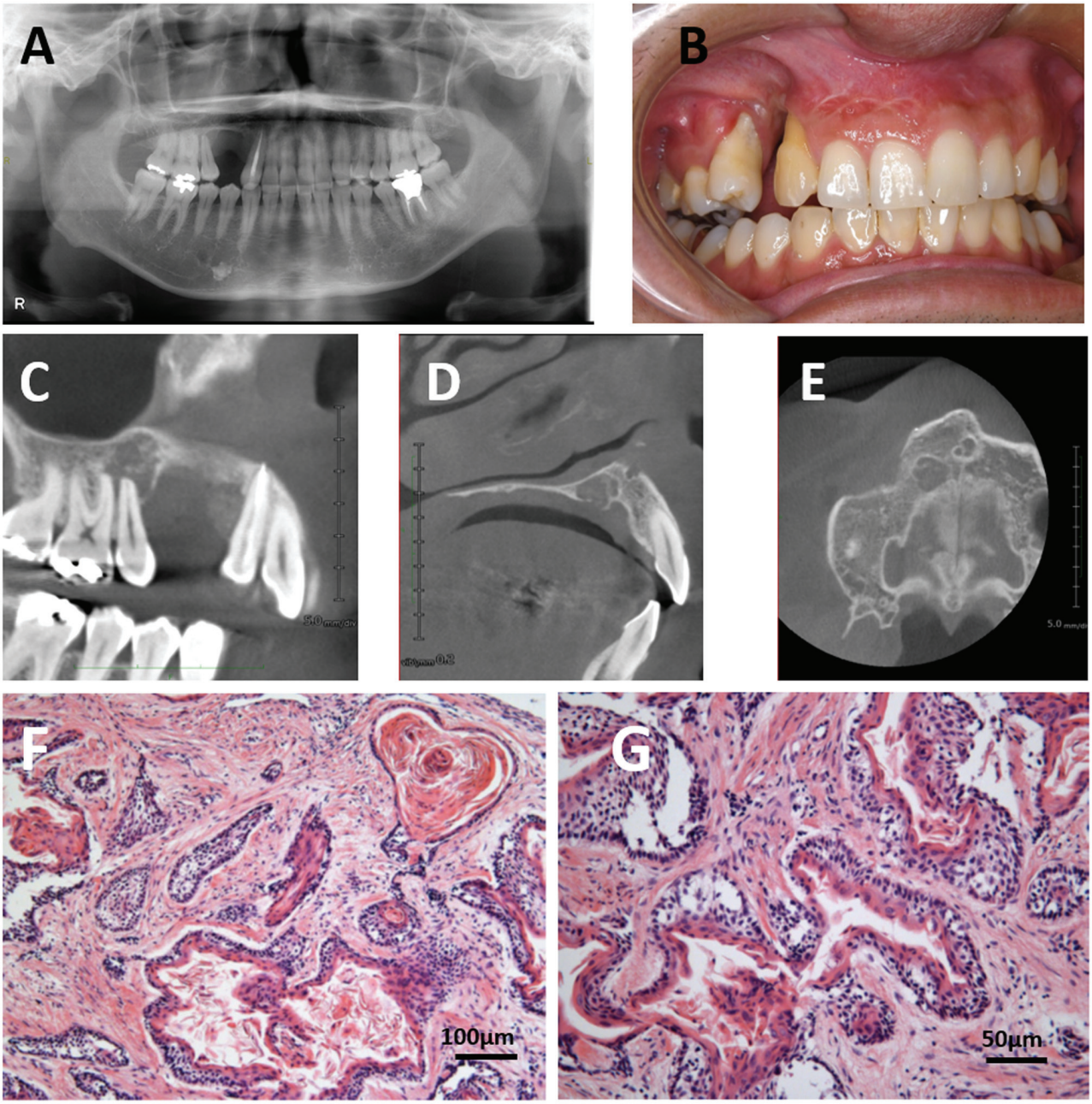

Figure 2. Recurrent tissue diagnosed as SOKC/KA. (A) Panoramic photograph before treatment. (B) Intraoral photograph before treatment. (C-E) Cone-beam CT imaging. (C) On the side of the maxillary sinus crest from the right second premolar root apex and (D) on the right canine tooth palatal side, a lesion with well-defined but slightly uneven margins was observed, (E) some of which were in close proximity to the nasal floor. (F and G) Histopathological analysis of SOKC/KA. Hematoxylin and eosin staining; (F) magnification, x100 and (G) magnification, x200. These samples consisted of a number of small cysts and solid epithelial nests with parakeratinization and central lamellated accumulation of keratin. Focal areas of loosely arranged polygonal epithelial cells resembling the stellate reticulum of the enamel organ and reverse nuclear polarity of the basal cells were observed. SOKC/KA, solid variant of odontogenic keratocyst/keratoameloblastoma.

recurrent lesions diagnosed as SOKC/KA. Data from the ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), CancerVar (https://cancervar.wglab.org/) and OncoKB™ (https://www.oncokb.org/) databases indicated that the detected mutation in *APC* (NM_000038:c.2626C>T; p.Arg876*) may be clinically pathogenic, the detected mutation in *KRAS* (NM_004985:c.38G>A; p.Gly13Asp) may be oncogenic, and the detected missense variant of *TP53* (NM_000546:c.91G>A; p.Val31Ile) may be clinically pathogenic. Other gene mutations that were detected are shown in Table I. In addition, since MAPK and mTOR are known to be activated downstream of *KRAS*, p-S6 and p-ERK1/2 expression was detected; IHC of the recurrent lesions showed focal positivity for p-S6 and p-ERK1/2 in the tumor nests (Fig. S1).

Discussion

In the present case, a primary unicystic lesion was readily diagnosed as OKC based on the characteristic lining of a thin and flat parakeratotic squamous epithelium composed of a palisaded basal layer and a corrugated surface. The complex histopathology of the recurrent lesions, which consisted of a solid proliferation of

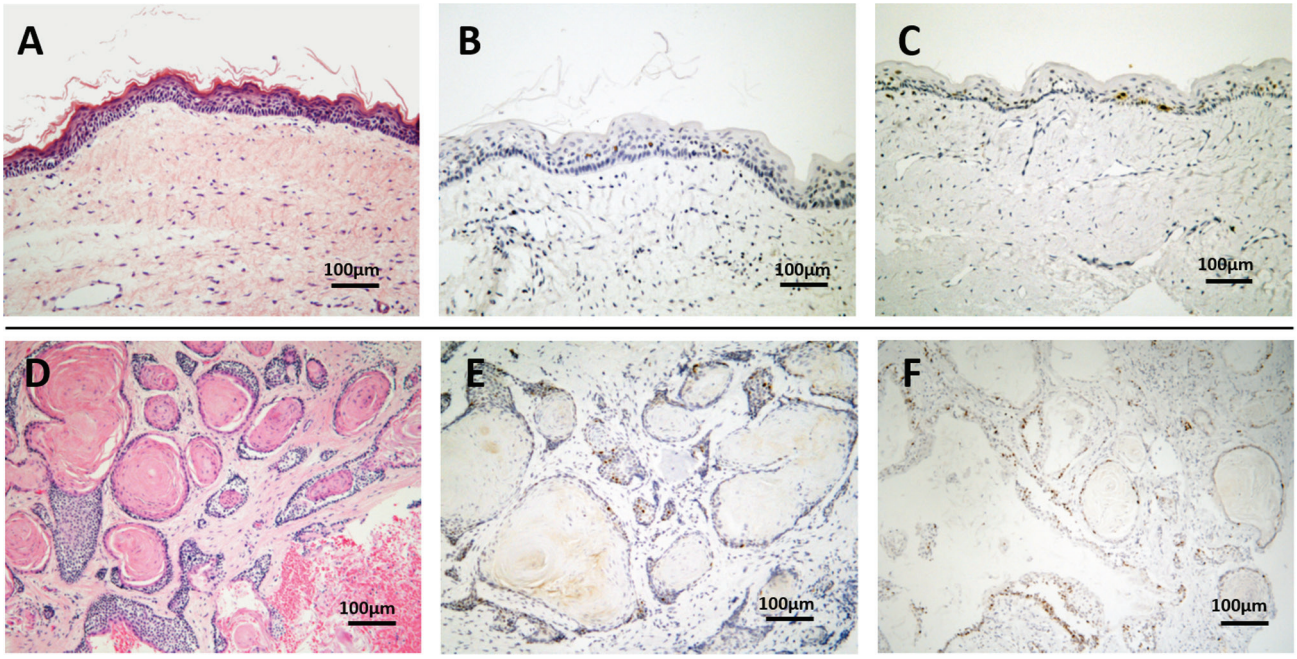

Figure 3. H&E staining and immunohistochemistry of (A-C) primary and (D-F) recurrent tissues (10 years after the initial diagnosis). (A and D) H&E staining. (B and E) Immunohistochemistry of p53. (C and F) Immunohistochemistry of Ki-63. Although the staining for p53 in recurrent lesions was slightly higher than in primary lesion and the Ki-67 labeling of the epithelial cells in the recurrent lesions (~10%) was higher than that in the primary lesion (~4%), the cellular and nuclear pleomorphism was not prominent in every lesion. Magnification, x100. H&E, hematoxylin and eosin.

numerous small cysts and epithelial islands with prominent keratinization, stellate reticulum-like configuration and subnuclear vacuolization of basal cells, complicated the diagnosis. Although the proliferative activity in the recurrent lesions was higher than that in the primary lesion, malignant transformation was ruled out due to the absence of cellular atypia.

In the 2000s, SOKC, an extremely rare form of OKC, was identified (4,5). SOKC is macroscopically solid and microscopically composed of multiple keratinizing microcysts and epithelial islands with central keratinization, each resembling OKC, in collagenous stroma. SOKC reportedly has aggressive clinical and radiographic features, including multilocular appearance, cortical expansion, infiltration into the bone marrow and soft tissues, and a tendency to recur (4-9). Based on these characteristics, OKC may consist of a spectrum of clinicopathological features from simple individual cysts, to cysts with multiple daughter cysts/epithelial islands, and to solid lesions recognized as true benign neoplasms. However, SOKC remains poorly defined because of unclear histopathological criteria due to the small number of reported cases (2,3,5,7,10). Given their overlapping pathological features, SOKC is difficult to distinguish from KA, a rare variant of AB with extensive keratinization in epithelial islands. Various histopathological features have been reported in cases of KA: i) Simple histology (follicular AB with extensive keratinization); ii) simple histology with OKC-like features; and iii) complex histology (simple histology with OKC-like features, epithelial follicles packed with parakeratin, and epithelial ribbons forming lamellar stacks of parakeratin extruded into the stroma) (10). The diagnostic differences between KA and SOKC are the stellate reticulum-like appearance of focal areas, subnuclear vacuolization of basal cells and lamellated-type central keratinization, which are characteristic of KA (11). However, a lesion with histological features resembling those of both SOKC and KA has been reported under the name of SOKC with ameloblastomatous transformation (7), and SOKC and KA may fall into a similar histological spectrum of odontogenic tumors. Ide *et al* (10) also suggested that SOKC and KA share a histogenetic relationship and form a clinicopathological spectrum, indicating that they should not necessarily be separated into different entities. Therefore, the term 'SOKC/KA' seemed appropriate for the diagnosis of recurrent lesions reported on in the present case report. The present case supports the idea that conventional OKC may be a histogenetic source of SOKC/KA, thus suggesting that a close histogenetic relationship exists among them.

Given the high recurrence rate in the present case, a genetic mutation analysis was performed. SNVs/InDels (170 genes) and fusions (25 genes) were analyzed in the recurrent lesions diagnosed as SOKC/KA. Data from the ClinVar, Cancer Var and OncoKB databases indicated that the mutation in *APC* (NM_000038:c.2626C>T; p.Arg876*) may be clinically pathogenic. Moreover, c.1488A>T; p.Thr496Thr may be a synonymous variant detected in exon 12 of *APC* in this case. *APC* is a multifunctional tumor suppressor gene that not only regulates β-catenin degradation in the Wnt signaling pathway but also controls cytoskeletal movement, regulates the cell cycle, and influences cell proliferation and division (12). Activation of the Wnt pathway is also important for tumor initiation and development, and mutations in *APC* cause regulatory dysfunction, which is closely linked to tumor initiation and development (12). Defects in APC induce β-catenin accumulation in the nucleus, leading to activation of the transcription factors TCF and LEF, which consequently activate the classical Wnt/β-catenin/TCF signaling pathway (12).

The present case report identified mutations in *KRAS* G13D, a mutation frequently detected in colorectal cancer (13). This

Table I. Results of the cancer panel gene analysis.

			ToMMo						Clinical significance			
Gene	Transcript ID (exon ID)	DNA change/ Protein change	38KJPN AF	gnomAD global AF	gnomAD EAS AF	gnomAD PopMax AF	AF, % (Alt/Total)	Exonic effect	Axen Cancer Panel 2 report	CancerVar	ClinVar	OncoKB
APC	NM_000038 (16/16)	c.2626C>T; p.Arg876*	None	None	None	None	4.8 (110/2,303)	Stop gain	Pathogenic	Pathogenic	Pathogenic	Likely oncogenic, likelyloss-of-function
RUNX1	NM_001754 (9/9)	c.1270T>G; p.Ser424Ala	0.004919	0.0002	0.000936	0.001702	11.7 (25/214)	Missense variant	Likely pathogenic	Pathogenic	Likely pathogenic	Unknown oncogenic effect
NTRK3	NM_001012338 (3/20)	c.61G>T; p.Val21Phe	0.014036	0.000291	0.005068	0.005068	33.2 (265/797)	Missense variant	Likely benign	Benign	Likely benign	Unknown oncogenic effect
APC	NM_000038 (12/16)	c.1488A>T; p.Thr496Thr	0.001628	0.00023	0.00672	0.00672	34 (803/2360)	Synonymous variant	Benign/likely benign	Benign	Benign/likely benign	Unknown oncogenic effect
IDH2	NM_002168 (7/11)	c.939A>G; p.Gly313Gly	0.06619	0.002263	0.055898	0.055898	24.5 (551/2,253)	Synonymous variant	Benign/likely benign	Benign	Benign/likely benign	Unknown oncogenic effect
STK11	NM_000455 (8/10)	c.1062C>G; p.Phe354Leu	0.045247	0.00396	0.040749	0.040749	25.8 (628/2437)	Missense variant	Benign/likely benign	Benign	Benign/likely benign	Unknown oncogenic effect
RUNX1	NM_001754 (9/9)	c.1415T>C; p.Leu472Pro	0.00983	0.000079	0.002138	0.002138	33.8 (71/210)	Missense variant	Benign/likely benign	Benign	Benign	Unknown oncogenic effect
MAP3K1	NM_005921 (1/20)	c.233_234delTCinsCT; p.Leu78Pro	0.007529	0.001662	0.004073	0.011671	44.6 (95/213)	Missense variant	Benign	-	Benign	Unknown oncogenic effect
NOTCH4	NM_004557 (1/30)	c.36_47delGCTGCTGCT GCT; p.Leu13_Leu16del	0.163822	0.108437	0.175256	0.175256	98.2(496/505)	Disruptive inframe deletion	Benign	-	Benign	Unknown oncogenic effect
ROS1	NM_001378902 (9/43)	c.883+3A>G; -	0.012953	0.001604	0.037943	0.037943	35.7 (616/1,725)	Splice variant intron variant	Benign	-	Benign	Unknown o ncogenic effect
SMO	NM_005631 (3/12)	c.582A>G; p.Glu194Glu	0.065299	0.003659	0.090979	0.090979	24.7 (406/1,646)	Synonymous variant	Benign	Unknown or conflict	Benign	Unknown oncogenic effect
NTRK2	NM_006180 (3/19)	c.249C>T; p.Asn83Asn	0.011311	0.000105	0.002699	0.002699	31.2 (681/2,184)	Synonymous variant	Benign	Unknown or conflict	Benign	Unknown oncogenic effect
PTEN	NM_000314 (1/9)	c.-366delT; -	0.999522	0.99926	0.99842	0.999738	99.1 (453/457)	5' UTR variant	Benign	-	-	Unknown oncogenic effect
PTEN	NM_000314 (1/9)	c.-326G>C; -	0.999341	0.999921	1.00000	1.000000	100 (319/319)	5' UTR variant	Benign	-	Benign	Unknown oncogenic effect
CDKN1B	NM_004064 (1/3)	c.165G>A; p.Ala55Ala	0.052425	0.001426	0.02812	0.02812	51.4 (636/1,237)	Synonymous variant	Benign	Unknown or conflict	Benign	Synonymous mutation
BRCA2	NM_000059 (11/27)	c.2350A>G; p.Met784Val	0.095501	0.000611	0.017514	0.017514	39.5 (736/1,865)	Missense variant	Benign	Benign	Benign	Conflicting (inconclusive)
BRCA2	NM_000059 (18/27)	c.8187G>T; p.Lys2729Asn	0.01728	0.00046	0.009808	0.009808	14.8 (399/2,687)	Missense variant	Benign	Pathogenic	Benign	Likely neutral
RNF43	NM_017763 (6/10)	c.597G>A; p.Val199Val	0.034683	0.000815	0.010978	0.010978	46.5 (435/936)	Synonymous variant	Benign	Unknown or conflict	Benign	Unknown oncogenic effect
ALK	NM_004304 (3/29)	c.941A>G; p.Glu314Gly	0.001201	0.000007	0.000193	0.000193	15.4 (186/1,206)	Missense variant	VUS	Benign	VUS	Synonymous mutation
RAD50	NM_005732 (12/25)	c.1924T>G; p.Leu642Val	0.003913	0.000046	0.001346	0.001346	50.2 (710/1,415)	Missense variant	VUS	Pathogenic	VUS	Unknown oncogenic effect
ERBB2	NM_004448 (27/27)	c.3430G>C; p.Asp1144His	None	None	None	None	37.6 (183/487)	Missense variant	VUS	Benign	VUS	Unknown oncogenic effect
KRAS	NM_004985 (2/5)	c.38G>A; p.Gly13Asp	None	0.00002	None	0.000044	10.3 (189/1,834)	Missense variant	Conflicting interpretations of pathogenicity	Pathogenic	Conflicting interpretations of pathogenicity: Pathogenic (6); uncertain significance (1)	Oncogenic/gain of function

Table I. Continued.

Gene	Transcript ID (exon ID)	DNA change/ Protein change	ToMMo				AF, % (Alt/Total)	Exonic effect	Clinical significance			
			38KJPN AF	gnomAD global AF	gnomAD EAS AF	gnomAD PopMax AF			Axen Cancer Panel 2 report	CancerVar	ClinVar	OncoKB
BRCA2	NM_000059 (10/27)	c.964A>C; p.Lys322Gln	0.011909	0.000079	0.002307	0.002307	48.5 (544/1,122)	Missense variant	Conflicting interpretations of pathogenicity	Pathogenic	Conflicting interpretations of pathogenicity: Likely pathogenic (1); uncertain significance (2); benign (4); likely benign (9)	Unknown oncogenic effect
TP53	NM_000546 (3/11)	c.91G>A; p.Val31Ile	0.00643	0.000099	0.002901	0.002901	8.6 (31/361)	Missense variant	Conflicting interpretations of pathogenicity	Pathogenic	Conflicting interpretations of pathogenicity: Likely pathogenic (1); uncertain significance (2); benign (1); likely benign (8)	Unknown oncogenic effect
STK11	NM_000455 (6/10)	c.842C>T; p.Pro281Leu	0.012138	0.000125	0.002499	0.002499	21.6 (269/1,248)	Missense variant	Conflicting interpretations of pathogenicity	Benign	Conflicting interpretations of pathogenicity: Uncertain significance (3); benign (3); likely benign (6)	Unknown oncogenic effect

VUS, variant of uncertain significance; AF, allele frequency; Alt, alternative; EAS, East Asian-specific; ToMMo, Japanese Tohoku Medical Megabank Organization.

mutation is considered the reason why the EGFR inhibitor cetuximab is highly effective for colorectal cancer, and the *KRAS* G13D mutation results in constant activation of the RAS/MAPK and MEKK/SEK/JNK pathways, allowing cancer cells to continue to invade and proliferate regardless of cell surface EGF stimulation (14). To the best of our knowledge, no *KRAS* G13D mutations in benign tumors arising from the oral cavity have been reported. However, adjacent *KRAS* G12V/R mutations have been reported in adenomatoid odontogenic tumor and AB in the oral cavity (15,16). In the present case, a *KRAS* mutation was detected, and the results of IHC showed focal positivity for p-S6 and p-ERK1/2 in the tumor nests. These results suggested that *KRAS* aberrations may activate downstream signaling and result in positive staining of p-S6 (16), which may reflect abnormal cell proliferation due to genetic mutations detected in the gene panel. In a previous case, the time to relapse of the SOKC/KA lesion was short, and an increased Ki-67 nuclear reaction in areas of cytologic atypia was observed, which may suggest possible malignant transformation, or proliferation of cells to form new neoplastic follicles and nests (17).

Treatment for AB includes jaw osteotomy with an emphasis on cure, and conservative surgical treatment aimed at preserving oral function, such as enucleation and fenestration. In the latter treatment, bone surface removal or cryotherapy may also be performed to increase the curative effect. SOKC/KA has been suggested to be more aggressive than purely cystic cases due to its infiltrative growth pattern and strong tendency to recur after resection (6). In a previous report, the mean recurrence rates for SOKC and KA cases were 12.5 and 41.7%, respectively, depending on the treatment method (3). In this previous study, SOKC/KA was reported to be treated similarly to AB, with conservative surgical therapy as the initial treatment in numerous cases. In the present case, conservative surgical therapy and bone surface removal were performed to preserve function in all procedures due to the patient age and extent of the lesion. Among the six cases of SOKC/KA (3) in which enucleation was performed, as in the present case, recurrence was observed in one of the five cases in the mandible and in the one case in the maxilla. Since SOKC/KA has been suggested to be more invasive than pure cystic cases, maxillary resection should also be considered. However, in the present case, the patient was young, and preferred enucleation and fenestration as a conservative surgical treatment, due to concerns about aesthetics and reduced quality of life. If clinical and imaging findings suggest a shorter recurrence period or malignant transformation, we plan to perform immediate radical treatment, such as jaw osteotomy.

The present study describes the case of a unicystic OKC that transformed into a hyperkeratotic SOKC/KA during long-term follow-up. The recurrent lesion was solid and consisted of OKC, AB with prominent central keratinization, and epithelium with features of both. Recently, the concept of SOKC/KA has been proposed based on the clinicopathological similarity between the two lesions (10,11). In accordance with this concept, the lesion in the present case was diagnosed as originating from OKC and becoming SOKC/KA upon recurrence. Notably, the immunohistochemical staining of calretinin, CD56, β-catenin and BRAFV600E was negative in the recurrent lesions, unlike in AB, and mutations in *APC* and *KRAS* were observed. Moreover, no mutations were observed in *BRAF* and *SMO*, which are generally common in OKC and AB. Thus, the clinical course

and histological transformation suggested the possibility of a novel subtype. In the present study, genomic DNA could not be extracted from the primary lesion and compared with recurrent lesions because of the long clinical course of the disease. In the future, it may be necessary to perform sequencing analysis at an earlier stage in recurrent cases to investigate *APC* and *KRAS* mutations.

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Availability of data and materials

The sequencing data generated in the present study may be found in the DDBJ BioProject database under accession number (DRR519302) or at the following URL: https://ddbj.nig.ac.jp/resource/biosample/SAMD00664639. The other data generated in the present study may be requested from the corresponding author.

Authors' contributions

SaY, TS and TA conceived the case presentation and drafted the manuscript. SaY, TS and SoY participated in the treatment of the patient. TA and MM performed pathological analysis. SaY and TS confirm the authenticity of all the raw data. All authors contributed to the discussion and critical comments. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from this patient, including consent to participate. Gene analysis was approved by the Ethics Committee of Hiroshima University (approval number: hi-72).

Patient consent for publication

Written informed consent was obtained from this patient, including consent for publication of the findings.

Competing interests

The authors declare that they have no competing interests.

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