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CASE REPORT

Overexpressed tumor protein p53 and the impaired function in aggressively growing calcifying odontogenic cyst: a case report

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ABSTRACT : A case of a calcifying odontogenic cyst (COC) is described. The patient was referred to our hospital for the treatment of a recurrence of an ameloblastoma. To perform enucleation as conservative surgery, deflation and biopsy were applied. From the specimens, the pathological diagnosis had changed to COC. After the biopsy, the lesions presented rapid growth and partial maxillectomy was performed. Morphologically, malignant findings were not determined in the surgical specimens. To exclude the possibility of malignant transformation because of the rapid growth, an immunohistochemical analysis with cell growth marker Ki-67 and tumor suppressor p53 was performed, here a somewhat high positive staining of 10 % for Ki-67 and 20-30 % for p53 were observed. To examine the metabolism and function of p53, further immunohistochemical staining was performed and showed negative staining for the p53 degradation enzyme MDM2 and positive staining of the p53-mediatied apoptosis molecule BAX with 80 % positive staining. These findings suggest that the lesion had a high growth potential as well as abnormal p53 metabolism and function. Finally, COC was assigned as the pathological diagnosis giving priority to the morphological findings. No recurrence has been observed for 3 years after resection.

Key Words : Calcifying odontogenic cyst, Calcifying cystic odontogenic tumor, p53, MDM2, BAX

Introduction

The COC is a rare odontogenic cyst which is characterized as a simple cyst with a well-defined basal layer, lined by ameloblastoma-like epithelium and with accumulations of ghost cells. It is not completely agreed whether the COC is to be classified as a tumor or as a developmental cyst[1]. The COC is also known as an odontogenic ghost cell lesion in conjunction with dentinogenic ghost cell tumors or ghost cell odontogenic carcinomas (GCOC)[1]. The criteria for a differential diagnosis as COC or GCOC are mainly based on morphological characteristics. An immunohistochemical analysis by p53 and PCNA to distinguish benign from malignant lesions has also been reported[2].

The p53 is a well studied tumor suppressor protein with the transcription ability to maintain cells under stress from DNA damage[3, 4]. Upon DNA damage, p53 functions as a transcription factor and activates various molecules related to cell cycles and apoptosis to protect cells from genetic instability. In half of human cancers, p53 loses its function caused by genetic mutation or deletion[5]. Many mutated or deleted p53 proteins, so called mutant p53 accumulate in cells escaping from the degradation enzyme MDM2 the expression of which is also controlled by p53[4]. The mitochondrial mediated apoptosis molecule BAX is also activated by p53 in cells in which where the accumulation of DNA damage is irreparable[6].

In this report, overexpressed p53 and its downstream protein, MDM2 and BAX in COC with rapid growth potential is described.

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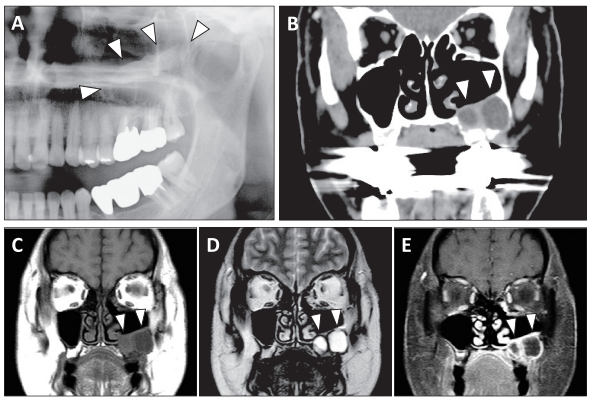

Fig. 1 Image analysis from the initial visit.
(A) Radiopaque lesion (arrowhead) identified in the left maxilla in the panoramic radiograph.
(B)–(E) Well enhanced multi-cystic lesion identified in the left upper alveolar region (arrowhead) in the computed tomography (CT) image (B), T1-weighted magnetic resonance (MR) image (C), T2-weighted image (D) and T1-weighted enhanced image (E).

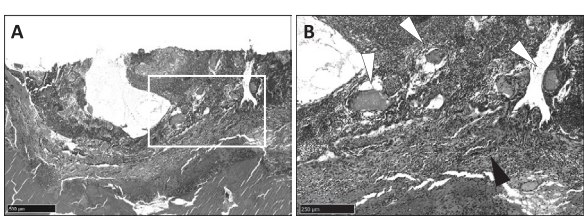

Fig. 2 Pathological findings in the biopsy specimen.
(A) Cyst with well-defined basal layer, lined by ameloblastoma -like epithelium (Hematoxylin and eosin, original magnification X 5). White rectangle in A is shown as a magnified area in B.
(B) Ameloblastomatous epithelium (white arrowhead) with scattered nests of ghost cells (black arrowhead), original magnification X 10.

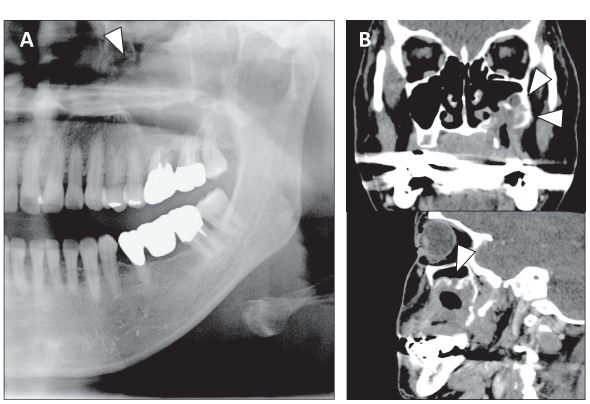

Fig. 3 Rapid growth of tumor after deflation and biopsy.
(A) Radiopaque lesion expanded in the panoramic radiograph.
(B) Multi-cystic lesions occupy the left maxilla sinus notably in the lateral and posterior regions.

Case report

A 50-year-old female had been aware of a swelling on her left cheek and stuffy nose. She had visited ENT surgeons and been diagnosed with an odontogenic cyst. After enucleation through the nasal cavity with endoscopic sinus surgery, the pathological diagnosis assigned it as an ameloblastoma. Six months after the treatment, there was a swelling and spontaneous pain in the left cheek. The ENT surgeons determined a recurrence of the tumor, and she was referred to our department for further treatment. A computed tomography (CT) and Magnetic resonance (MR) images showed a multicystic lesion localized in the left upper alveolar bone and floor of the maxillary sinus (Fig. 1). The nasal wall of the maxillary sinus had been removed as the ENT surgeon approached the lesion via the nasal cavity. Enucleation after deflation was planned to remove the recurrent ameloblastoma, and the specimens obtained by the deflation showed a cystic lesion lined by ameloblastomatous epithelium with scattered nests of ghost cells in the histological examination. Ameloblastic follicles and odontogenic islands were present in the adjacent connective tissue (Fig. 2). The pathological diagnosis was changed to COC, then, after deflation the lesion showed rapid growth to occupy the posterior wall of the sinus, and next partial maxillectomy was performed rather than enucleation (Fig. 3). Because of the rapid expansion of the tumor, an immunohistochemical (IHC) analysis was performed for a differential diagnosis to exclude a malignant transformation. No extensive necrosis was observed in a hematoxylin eosin stained specimen. An IHC analysis showed the growth marker Ki-67 (MIB-1 clone; Dako) as positive in 10 % of the tumor cells and p53 (DO-7 clone; Roche) in 20 to 30 % (Fig. 4 A, B). To determine the mechanism of the positive staining of p53, a further immunohistochemical analysis was performed against BAX (A3533 clone; DAKO) and MDM2 (IF2 clone; Invitrogen). The BAX is transcriptionally activated by p53 and causes apoptosis of the cells, and MDM2 is also activated by p53 and plays an important role in the degradation of p53, a so called negative feedback mechanism. In the IHC analysis, 80 % of the tumor cells were positive for BAX and there was negative staining in MDM2 (Fig. 4 C, D). Morphologically, there was no excessive mitosis or apoptosis, which would have indicated an absence of a malignant transformation. Finally, COC was diagnosed by the morphological

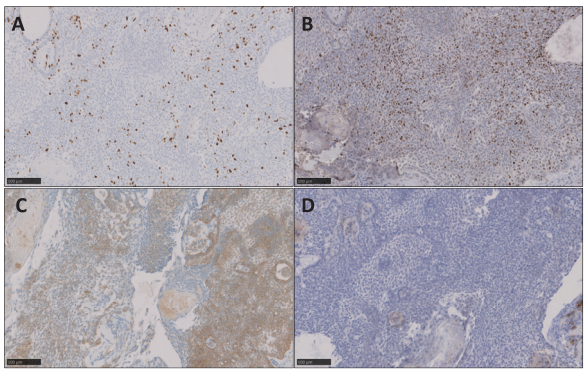

Fig. 4 IHC analysis of resected specimens.
Tumor cells showing positive staining of Ki-67 (A), p53 (B) and BAX (C). Negative staining of MDM2 (D), Original magnification X 20.

features. No recurrence has been observed for 3 years after the resection.

Discussion

In a recent version of WHO classifications, COC is considered as a developmental cyst, but it is also thought to be an odontogenic ghost cell lesion with dentinogenic ghost cell tumors or ghost cell odontogenic carcinomas (GCOC)[1]. The GCOC is the most infrequent ghost cell lesion with a wide variety of growth patterns[1]. Almost half of GCOC arise from pre-existing benign lesions by malignant transformation[7]. Positive staining of p53 is observed in two thirds of GCOC[7-9]. In addition to the regular cytological index including mitotic activity and pleomorphism, rapid growth and positive staining of p53 in IHC are practical indicators in a differential diagnosis. To the best knowledge of the authors, there is no report investigating the relations between p53 with its related molecules in COC.

The p53, a so called guardian of the genome, is a well investigated transcriptional factor, due to its important function in the maintenance of genomic stability[4]. Responding to DNA damage by UV, irradiation, hypoxia, or other metabolic stresses, p53 functions as a transcription factor and activates the expression of important molecules to save cells by maintaining their normal mitosis[3, 4]. The TP53 gene encoding p53 protein is mutated in more than half of cancers and the mutated region is accumulated in its DNA-binding domain which is important for its transcriptional activity[5]. A mutation or deletion, a major anomaly of the TP53 results in producing abnormal p53 and accumulation of abnormal p53 in the cells.

The half-life of normal, so called, wild type p53 is strictly controlled by its degradation enzyme molecule MDM2[10]. The expression of MDM2 is dependent on the transcriptional activity of wild type p53, so positive staining of p53 in IHC analysis generally indicates the mutation or impaired functioning of p53[11]. The p53 is also responsible for cell apoptosis by activating BAX which plays an important role in inducing mitochondria mediated apoptosis in the cells in which the accumulation of DNA damage is irreparable[6].

In the present report, a COC in the left maxilla which showed rapid growth after a biopsy is described. At the first visit, enucleation was scheduled assuming a recurrence ameloblastoma. But by the rapid growth of the lesion after the biopsy and by altered pathological diagnosis to COC of the biopsy specimens, operation was changed to partial maxillectomy to remove the lesion completely. Differential diagnosis had been performed to exclude its malignant potential after complete resection. In the IHC analysis against Ki-67 and p53, tumor cells showed positive staining of Ki-67 and p53. These results indicated a strong growth potential by the Ki-67 staining and the possibility of malignant transformation by accumulation of p53. After further IHC analysis was performed to evaluate the p53 metabolisms and function by an IHC analysis against MDM2 and BAX, the expression of which is controlled by p53. Results of the IHC analysis showed negative staining of MDM2 and positive staining of BAX.

In Fig. 4B, the positive staining of p53 in the IHC assumes the accumulation of mutant p53 escaping from degradation by MDM2[10]. The discrepancy in the expression levels of both p53 transactivating molecule MDM2 and BAX may implicate either the anomalous transcriptional activity of mutant p53[12], or ARF-dependent degradation of MDM2 induced by wild type p53-mediated cell-cycle arrest[13].

Inordinate apoptosis was not observed in histopathological specimen despite of positive staining of BAX in the IHC (Fig. 4). There is a report that benign lesion such as ameloblastoma, odontogenic keratocyst or radicular cyst expresses BAX[14]. Executing the apoptosis, BAX forms a complex with other apoptosis related factor, so called Bcl-2 family[6]. Further studies on the apoptosis related molecule in benign odontogenic lesions may help for better understanding of the role of BAX in COC.

Finally, the pathological diagnosis of COC was assigned based on the morphological findings without atypia, massive mitosis, or apoptosis. No recurrence has been observed for 3 years after the resection.

Conflict of interest

Authors have no conflicts of interest to declare.

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Reference

1) Ledesma-Montes C, Gorlin RJ, Shear M, Torius FP, Mosqueda-Taylor A, Atlini M, Unni K, Paes de Almeida O, Carlos-Bregni R, Romero de León E, Phillips V, Delgado-Azañero W, Meneses-García A : International collaborative study on ghost cell odontogenic tumours : calcifying cystic odontogenic tumour, dentinogenic ghost cell tumour and ghost cell odontogenic carcinoma. J Oral Pathol Med 37 : 302-308, 2008.
2) Saghafi S, Zare-Mahmoodabadi R, Salehinejad J, Kadeh H, Afzal-Aghaee M : Immunohistochemical analysis of p53 and PCNA expression in calcifying odontogenic cyst. J Oral Sci 52 : 609-613, 2010.
3) Vogelstein B, Lane D, Levine AJ : Surfing the p53 network. Nature 408 : 307-310, 2000.
4) Lane DP. Cancer : p53, Guardian of the Genome. Nature 358 : 15-16, 1992.
5) Vousden KH, Lane DP : p53 in health and disease. Nat Rev Mol Cell Biol 8 : 275-283, 2007.
6) Chipuk JE, Kuwana T, Bouchier-Hayes L, Droin NM, Newmeyer DD, Schuler M, Green DR : Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. Science 303 : 1010-1014, 2004.
7) Del Corso G, Tardio ML, Gissi DB, Marchetti C, Motebugnoli L, Tarsitano A : Ki-67 and p53 expression in ghost cell odontogenic carcinoma : a case report and literature review. Oral Maxillofac Surg 19 : 85-89, 2015.
8) Lu Y, Mock D, Takata T, Jordan RC : Odontogenic ghost cell carcinoma : report of four new cases and review of the literature. J Oral Pathol Med 28 : 323-329, 1999.
9) Motosugi U, Ogawa I, Yoda T, Abe T, Sugasawa M, Murata S, Yasuda M, Sakurai T, Shimizu Y, Simizu M : Ghost cell odontogenic carcinoma arising in calcifying odontogenic cyst. Ann Diagn Pathol 13 : 394-397, 2009.
10) Chuikov S, Kurash JK, Wilson JR, Xiao B, Justin N, Ivanov NJ, McKinney K, Tempst P, Prives C, Gamblin SJ, Barlev NA, Reinberg D : Regulation of p53 activity through lysine methylation. Nature 432 : 353-360, 2004.
11) Bond GL, Hu W, Levine AJ : MDM2 is a central node in the p53 pathway: 12 years and counting. Curr Cancer Drug Targets 5 : 3-8, 2005.
12) Kato S, Han SY, Liu W, Otsuka K, Kanamaru K, Ishioka C : Understanding the function-structure and function-mutation relationships of p53 tumor suppressor protein by high-resolution missense mutation analysis. Proc Natl Acad Sci U S A 100 : 8424-8429, 2003.
13) Zhang Y, Xiong Y, Yarbrough WG : ARF promotes MDM2 degradation and stabilizes p53 : ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways. Cell 92 : 725-734, 1998.
14) Soluk Tekkeşın M, Mutlu S, Olgaç V : Expressions of bax, bcl-2 and Ki-67 in odontogenic keratocysts (Keratocystic Odontogenic Tumor) in comparison with ameloblastomas and radicular cysts. Turk Patoloji Derg 28 : 49-55 : 2012.