A case of salivary duct carcinoma possibly arising from pleomorphic adenoma

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Abstract

Objective: We report a rare case of salivary duct carcinoma possibly arising from the malignant component of carcinoma ex pleomorphic adenoma.

Material and Methods: A 56-year-old Japanese male complained of swelling in the left side of jaw for 2 years. On extraoral examination, the face was bilaterally asymmetrical. An ultrasonography revealed hypoechoic mass of size 1.5x2.5x2.5 cm on left parotid gland. Computed tomography (CT) revealed a slight irregular mass on left parotid gland with no infiltration to adjacent tissues. Based on these findings, a clinical diagnosis of pleomorphic benign tumor was made.

Results: A surgical excision was performed under general anesthesia. On histopathological examination, the connective tissue was rich in fibrous components and was accompanied by hyaline. Epithelial cells with high atypia proliferating invasively as cords into connective tissue were observed. The intraductal component had Roman-bridge-like architecture with central portion undergoing comedonecrosis. Based on these findings, a final diagnosis of salivary duct carcinoma possibly arising from pleomorphic adenoma was made. p16 hypermethylation is a known phenomenon in oral malignancy. We also checked the methylation status of p16 gene and found high level of methylation in this tumor.

Conclusion: We reported a rare case of salivary duct carcinoma possibly arising from pleomorphic adenoma and also demonstrated high methylation level of p16 gene in the tumor.

Keywords: Gene, Methylation, Parotid gland, Pleomorphic adenoma, P16, Salivary duct carcinoma

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Introduction

Salivary duct carcinoma is a malignant tumor of salivary gland. It is a rare tumor but presents as one of the most aggressive among malignant salivary gland tumors.1 It arises most commonly in the parotid gland followed by submandibular gland, minor salivary glands of oral cavity and larynx.2 The incidence of salivary duct carcinoma ranges from 1-3% of all salivary gland malignancies with male predilection affecting people over 50 years of age.3 It resembles histologically and immunohistochemically with high grade mammary ductal carcinoma.4

The salivary duct carcinoma is not frequently observed in a single center because of its rare nature. However, with growing multicenter studies, the clinical findings, histopathological and immunohistochemical profile have been defined. The majority of cases arises de novo while some cases arising from pleomorphic adenoma have been reported.5 Herein, we report a rare case of salivary duct carcinoma possibly arising from pleomorphic adenoma. Also, p16 hypermethylation is involved in oral malignancy including salivary gland tumors.6 We also checked the methylation status of the gene in the present case.

Case Report

A 56-year-old Japanese male was referred to Health Sciences University of Hokkaido Hospital with a chief complain of swelling in the left parotid region for 2 years. The swelling was painless until 2 days ago and was slow progressing. On clinical examination, the face was bilaterally asymmetrical. The mass was single, well-circumscribed on the lower lobe of the left parotid gland figure 1A. The overlying skin surface was normal in appearance. On intra oral examination, there were no abnormal findings. An ultrasonography revealed hypoechoic mass of size 1.5x2.5x2.5 cm on left parotid gland. Computed tomography (CT) revealed a slight irregular mass on left parotid gland with no infiltration to adjacent tissues figure 1B. Based on above-mentioned findings, a provisional clinical diagnosis of left parotid benign tumor was made. Surgical excision of the tumor mass was performed under general anesthesia figure 1C. The mass was immediately fixed in 10% formalin solution and sent for histopathological examination.

On histopathological examination, the sections showed that the connective tissue was rich in fibrous components and was accompanied by hyaline. Epithelial cells with high atypia
A CASE REPORT

proliferating invasively as cords into connective tissue were observed. The intraductal component had Roman-bridge-like architecture with central portion undergoing comedonecrosis figure 2A. A small part of tissue showed myxochondroid-like structures figure 2B. The surrounding area was covered with a capsule-like structure, and no obvious extracapsular invasion was observed.

Immunohistochemistry (IHC) was performed using antibodies against cytokeratin (CKAE1/AE3), p53, S-100, glial fibrillary acidic protein (GFAP), and epithelial membrane antigen (EMA). The cells were positive for CKAE1/AE3, an epithelial cell marker. Also, positive staining for EMA, a ductal cell marker was observed figure 2C. A few cells showed positive staining for GFAP, whereas no positive staining for S-100 was observed in the tumour nests. Some cells in few areas were positively stained with p53 figure 2D.

The genomic DNA was extracted from three different blocks of formalin fixed paraffin embedded tumor tissue using DNeasy Blood and Tissue kit (Qiagen, Germany). Five hundred ng of the DNA was subjected to bisulfite conversion using the EpTect Bisulfite Conversion kit (Qiagen, Germany). The methylated and unmethylated primers for each gene were designed using MethPrimer (https://urogene.org/methprimer/). The primer sequences used were as follows: [MF:TTTAGAATGTGGGATTATAGCT; MR:AAAAAATTTTAAACAAAATACTGCT; UF:TTTAGAATGTGGGATTATAGATGT UR:AAAAAACTAAAACAAAAAAATTCCT] Bisulfite-converted DNA, KAPA SYBR Fast qPCR kit (NIPPON Genetics, Japan) and a pair of methylated or unmethylated primers were mixed and subjected to quantitative methylation specific polymerase chain reaction (qMSP) in Light Cycler Nano System (Roche Diagnostics, Germany) under following conditions: denaturation at 95 °C for 10 min, 50 cycles of denaturation at 95 °C for 10 s, and annealing at 60 °C for 30 s.

The percentage of DNA methylation in each sample was estimated using the following formula:

\[ Methylated \ DNA \ (%) = \frac{M}{(M+U)} \times 100 = \frac{1}{(1+2^{-\Delta Cq})} \times 100 \]

where, M and U are the copy numbers of methylated and unmethylated DNA, respectively, and \( \Delta Cq = Cq_U - Cq_M \).

The p16 gene showed very high amount of methylation in all three specimens as evaluated by qMSP. The methylation level ranged from 92% to 97% in those specimens' figure 3.

Discussion

Herein, we reported a case of salivary duct carcinoma possibly arising from pleomorphic adenoma. We showed high methylation level of p16 in those tumor tissues. Salivary duct carcinoma is a rare type of salivary gland malignancy.1 The prevalence of salivary gland malignancy ranges from 1-3% of all head and neck malignancies and 0.3% of all malignant neoplasms.2 The salivary duct carcinoma comprises 1-3% of all salivary gland malignancies.3 It is estimated that salivary duct carcinoma arises de novo in 80% of the cases while approximately 20% arises from pleomorphic adenoma.3 In our case,
myxochondroid-like structures and a few GFAP positive cells were detected at the only limited part of the tissues. These findings support that the present case possibly arose from pleomorphic adenoma.

The malignant part of carcinoma ex pleomorphic adenoma is most often diagnosed as adenocarcinoma not otherwise specified.8,9 In some cases, it has been diagnosed as mucoepidermoid carcinoma, acinic cell carcinoma, adenoid cystic carcinoma, myoepithelial carcinoma and salivary duct carcinoma.9 In the present study, the intraductal component had Roman-bridge-like architecture with central portion undergoing comedonecrosis indicating it could be salivary duct carcinoma.1 Salivary duct carcinoma is the one of most aggressive tumors among all salivary gland malignancies.1 More than 50% of the patients are reported to die within 3-5 years with low overall survival (42% for stage I and 23% for stage IV).3,10 Therefore, early detection and close observation is crucial for better treatment outcome.

p16 gene hypermethylation has been observed in various salivary gland malignancies.6 p16, a tumor suppressor gene, is a cyclin D-dependent protein kinase inhibitor that regulates cell cycle arrest at G1 phase.11 The hypermethylation of p16 may lead to p16 silencing thereby promoting cell proliferation linked to carcinogenesis.11 In the present study, we demonstrated high methylation level (>90%) of p16 in all three specimens of salivary duct carcinoma. Our findings were consistent with the previous study which showed 60-90% methylation of p16 gene in malignant salivary tumor.6 Further studies using a normal salivary gland as a control are needed to fully understand the methylation level.

Conclusion

We reported a rare case of salivary duct carcinoma possibly arising from pleomorphic adenoma and also demonstrated high methylation level of p16 gene in the tumor.

Acknowledgment

None.

Conflict of Interest

The authors report no conflict of interest.

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Figure 3. Methylation level of p16 gene in the tumor. The p16 gene showed high methylation level (>90%) in all tissue specimens of the tumor as evaluated by qMSP. Data presented as mean ± standard error.