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Original Article

Different correlations between tumor size and cancer-related gene profiles according to histologic type of salivary gland tumor

Hyunsu Lee[1], Dongbin Ahn[2], Jin Ho Sohn[2], Yong-Hee Kim[3], Jae-Ho Lee[1], Heejin Kim[4]

[1]Department of Anatomy, Keimyung University School of Medicine, Daegu, South Korea; [2]Department of Otolaryngology-Head and Neck Surgery, Kyungpook National University Hospital, Daegu, South Korea; [3]Department of Microbiology, Kyungpook National University School of Medicine, Daegu, South Korea; [4]Department of Otorhinolaryngology-Head and Neck Surgery, Hallym University Dongtan Sacred Heart Hospital, Hwaseong, Kyeonggido, South Korea

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Abstract: Salivary gland tumors are mostly benign, and malignant tumors are rare. Because of this rarity, there is little molecular biology research on salivary gland tumors. Recently, we have published an analysis of the telomere length (TL) in salivary gland tumors. In this paper, we analyzed amplification of the catalytic subunit of phosphatidylinositol 3-kinase (PIK3CA) and mitochondrial DNA copy number (mtCN) in salivary gland tumors. To investigate mutations in PIK3CA, we performed genomic sequencing on samples of salivary gland tumors extracted from patients. The expression level of PIK3CA mRNA and mtCN were measured by RT-PCR. PIK3CA amplification and mtCN did not differ between Warthin's tumor (WT), pleomorphic adenoma (PA), and carcinoma of the salivary gland. The size of the tumor and the molecular profile correlated in three relationships: the size of WT with PIK3CA and with mtCN, and the size of PA with TL. We found no correlation between the size of carcinoma and the molecular profile. There was no correlation between age and molecular profile in all histologic groups of salivary gland tumor. We found no correlation between TL and mtCN in each histologic group. Although we have not found any significant results for the molecular profile of salivary gland tumors, our study can be a basis for further studies on other oncogenes in salivary gland tumors.

Keywords: Salivary gland tumor, telomere length, mitochondrial DNA copy number, PIK3CA

Introduction

Most salivary gland tumors are benign, and malignant tumors arising in the salivary gland are relatively rare [1, 2]. In the Korean population, the incidence of malignant tumors of the major salivary gland was 1.0 case per 100,000 population in 2014, which is only 0.2% of all malignancies and about 15% of cancers of the head and neck [3]. Mucoepidermoid carcinoma is the most common histologic type in the major salivary gland, followed by adenoid cystic carcinoma, adenocarcinoma, and acinic cell carcinoma. Among parotid tumors, the most common histopathology of benign tumor is pleomorphic adenoma (PA), followed by Warthin's tumor (WT) [4-7]. Among many studies, malignant transformation has been reported at a rate of 1.1~6.2% for PA [8-11], and 0.03% for all WT cases [12]. Due to the variety of pathology and rarity, little is known about the molecular profiles of salivary gland tumors.

Telomeres are repetitive sequences that protect chromosomes from shortening on cell division at both ends of the chromosome. Telomerase reverse transcriptase (TERT) is a telomerase subunit that regulates and maintains telomere length (TL). Recently, somatic mutations in the promoter region of αTERT have been found in various cancers including melanoma [13, 14], gastric cancer [15, 16], thyroid cancer [17], and urothelial cancer [18]. We recently reported that there is no correlation between histopathologic findings and TL, with little evidence of TERT promoter mutation in salivary gland tumors [19]. Mitochondrial DNA (mtDNA) is one of the other genes involved in tumorigen-

Table 1. Descriptive statistics according to the histologic group of salivary gland tumors

	Pleomorphic adenoma (N = 19)	Warthin's tumor (N = 8)	Carcinoma (N = 5)	*P* value
Sex				0.026
Male	6 (31.6%)	7 (87.5%)	3 (60.0%)	
Female	13 (86.4%)	1 (12.5%)	2 (40.0%)	
Age (years)	40.1 ± 11.1	64.1 ± 5.4	58.8 ± 7.3	< 0.005
Location				0.936
Parotid gland	16 (84.2%)	7 (87.5%)	4 (80.0%)	
Submandibular gland	3 (15.8%)	1 (12.5%)	1 (20.0%)	
Size (cm)	3.0 ± 1.2	3.5 ± 1.3	2.7 ± 0.9	0.476
Telomere length	6.2 ± 8.4	12.3 ± 25.5	3.1 ± 4.5	0.613
PIK3CA amplification	0.5 ± 0.3	0.6 ± 0.2	0.6 ± 0.3	0.428
mtDNA copy number	31.8 ± 85.4	1.1 ± 2.0	1.6 ± 1.2	0.252

esis. Recently, mitochondrial dysfunction has been suggested as a critical tumorigenesis pathway in human tumors, including salivary malignancy [20, 21]. We reported a relationship between TL and mtDNA copy number (mtCN) in colorectal cancer [22] and gastric cancer [23]. However, no studies have reported a relationship between TL and mtCN in salivary gland tumors.

Over the past decades, abnormalities of phosphatidylinositol 3-kinase (PI3K) signaling pathway have been evaluated in various human cancers including salivary gland malignancy [24-26]. The amplification or the mutation of the gene encoding the p110α catalytic subunit of PI3K (PIK3CA) has been examined as an oncogenic abnormality in various cancers [27]. The relevance of abnormality of PIK3CA in the occurrence of salivary gland tumor, however, is not fully characterized yet, especially in the Korean population.

We aimed to evaluate PIK3CA amplification and mtCN according to histologic type of salivary gland tumor. Also, the correlation between TL and mtCN was analyzed in salivary gland tumors. Based on the molecular differences of WT, PA, and carcinoma, we sought to reveal the underlying molecular mechanisms of malignant transformation.

Materials and methods

Patients

We performed a retrospective analysis of 32 cases of salivary gland tumor between November 2014 and May 2015. The institutional review board at the Kyungpook National University College of Medicine, Daegu, Korea, reviewed and approved the study protocol and exempted informed consent for this study (IRB no: 2014-09-002-001). We performed all procedures by the tenets of the World Medical Association's Declaration of Helsinki. All patients underwent salivary gland resection for their tumor, and their clinical data was collected from medical records. The histologic type of the collected tumor sample was confirmed by a pathologist (**Table 1**). Histologic types of thirty-two cases were divided into three groups: 19 PAs, 8 WTs, and 5 carcinomas. The five instances of carcinoma consisted of adenosquamous carcinoma, poorly differentiated adenocarcinoma, epithelial-myoepithelial carcinoma, adenoid cystic carcinoma, and salivary duct carcinoma. After curative resection, some part of the tumor was sampled for this study and underwent DNA extraction. DNA was isolated using an Absolute TM DNA Extraction kit (BioSewoom, Inc., Seoul, Korea), according to the manufacturer's protocol. DNA quantity and quality were measured using NanoDrop 1000 (Thermo Fisher Scientic, Inc., Pittsburgh, PA, USA).

PIK3CA mutation and amplification

As previously described [28], two hot spot-regions (exons 9 and 20) of PIK3CA mutation were investigated. PCR was done using AmpliTaq Gold (Applied Biosystems, USA). The PCR conditions were as follows: 1 cycle of 95°C for 11 min, 40 cycles of 95°C for 30 sec, 55°C for 40 sec, and 72°C for 1 min, followed by one

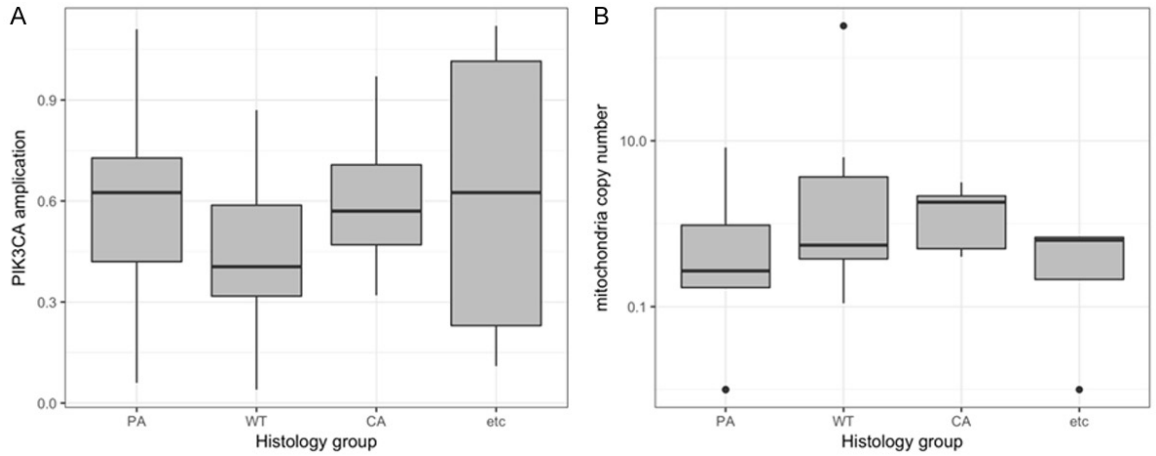

Figure 1. Box plots of the values including PIK3CA amplification (A) and mtCN (B) for each histology group. The vertical line extends from the smallest non-outlier to the largest non-outlier. The box was drawn from 25 percentiles to 75 percentiles. The thick horizontal line in the box means the median of the data. PA, pleomorphic adenoma; WT, Warthin's tumor; CA, malignant cancer; etc, another benign tumor.

cycle of 72°C for 10 min. Then, direct DNA sequencing for PIK3CA mutation was performed using the ABI 3730 DNA sequencer by Bionics Inc., Korea.

TL, PIK3CA amplification, and mtCN

Detailed methods for analyzing relative TL, PIK3CA amplification, and mtCN were previously described [22, 23]. Briefly, quantitative measurement of each gene was performed by quantitative real-time (qRT) PCR. Specific primers for telomeric repeats, PIK3CA, and cytochrome oxidase subunit I (COXI, a gene in mtDNA) were selected as previously described. qRT-PCR was performed on a LightCycler 480 II system (Roche Diagnostics, Germany). The relative values of target gene copies of β-actin or β-globin were calculated as previously described. Each measurement was repeated in triplicate, and five serially diluted control samples were included in each experiment.

Statistical analysis

R 3.4 and the ggplot2 package were used for statistical analysis and plotting [29, 30]. Pearson's Chi-square test, ANOVA test, Mann-Whitney U test, and Spearman's rank correlation analysis were used to analyze the relationship between variables as described in the results section. *P* values < 0.05 were considered significant, and all *p* values correspond to two-sided significance tests.

Results

PIK3CA amplification and mtCN were not different according to histologic characteristics of salivary gland tumors

We examined whether the characteristics of salivary gland tumors differ according to histologic characteristics. We used sequencing methods to investigate whether PIK3CA mutations and amplifications differ by histologic characteristics. Unfortunately, no PIK3CA mutation was found in any of the 32 cases. The mean and standard deviation of PIK3CA amplification were as follows by histologic type: 0.5 ± 0.3 for WT, 0.6 ± 0.2 for PA, and 0.6 ± 0.3 for carcinoma. A difference between histologic groups of the salivary gland tumor was not found in PIK3CA amplification (**Figure 1A**, ANOVA test, *P = 0.673*). We investigated whether the mtCN differs depending on the histologic characteristics of the salivary gland tumor. The means and standard deviation of mtCN were, by histologic type: 31.8 ± 85.4 for WT, 1.1 ± 2.0 for PA, and 1.6 ± 1.2 for carcinoma. Although investigation on the mtCN revealed that mtCN of WT was higher than other histologic groups, there was no statistical difference (**Figure 1B**, ANOVA test, *P = 0.353*).

Correlation of the size of salivary gland tumor with TL, PIK3CA amplification, mtCN

We investigated whether TL, PIK3CA amplification, and mtCN correlate with the size of the salivary gland tumor (**Figure 2**). Spearman's

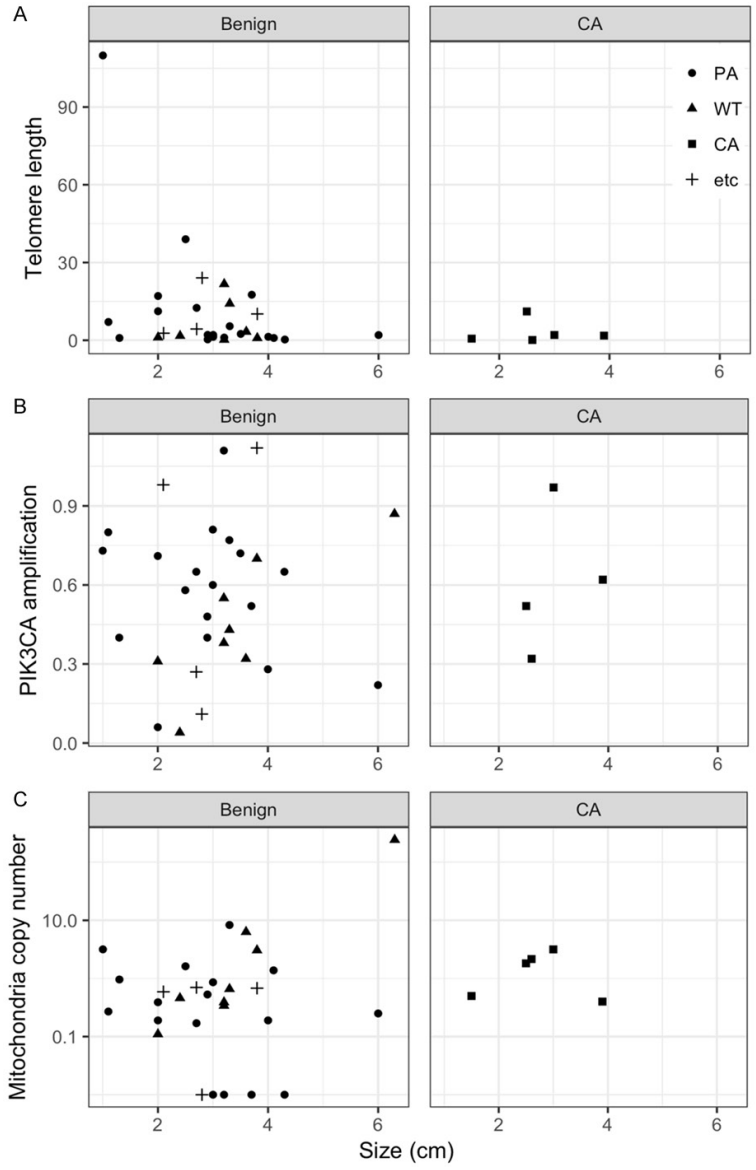

Figure 2. Scatter plot of telomere length (A), PIK3CA amplification (B), and mtDNA copy number (C) with tumor size according to histologic group of the salivary gland tumors. PA (closed circle), pleomorphic adenoma; WT (closed triangle), Warthin's tumor; CA (closed rectangle), carcinoma; mtCN, mtDNA copy number.

rank correlation analysis was performed separately for each of the three gene expression profiles for each of the three tumor groups; thus, nine correlational analyses were performed. The correlation between tumor size and TL was statistically significant in PA (P = *0.040*); whereas, PIK3CA amplification and mtCN correlated with tumor size in WT (P = *0.020* and *0.002*, respectively). Except for these, however, no correlation was observed.

No correlation between the age and TL, PIK3CA amplification, mtCN

We investigated whether TL, PIK3CA amplification, and mtCN correlate with the age of patients with salivary gland tumor. As with size analysis, Spearman's rank correlation analysis was performed. However, all of the correlation analyses were not statistically significant.

No correlation between TL and the mtCN of salivary gland tumor

We have reported correlations between TL and mtCN in colorectal cancer [22] and gastric cancer [23]. Thus, we investigated whether TL correlates with mtCN of the salivary gland tumor (**Figure 3**). As previously done, Spearman's rank correlation analysis was performed. However, there was no correlation between TL and mtCN in WT, PA, and carcinoma (P = *0.595, 0.399*, and *0.950*, respectively).

Discussion

Salivary gland tumors, including malignancy, represent a heterogeneous group of pathology, so it is difficult to understand their molecular pathogenesis and genetic alterations. As is well known, PA is the most common benign tumor of the parotid gland, and the World Health Organization classification reported that 3-4% of all pleomorphic adenomas become malignant [31]. Carcinoma ex-pleomorphic adenoma (CXPA) is defined as a carcinoma arising from a primary or recurrent PA [8, 32]. Unlike PA, Warthin's tumor presents less than a 1% risk of malignant transformation [12]. We collected 32 surgically treated salivary tumors and analyzed their clinicopathologic and molecular biologic

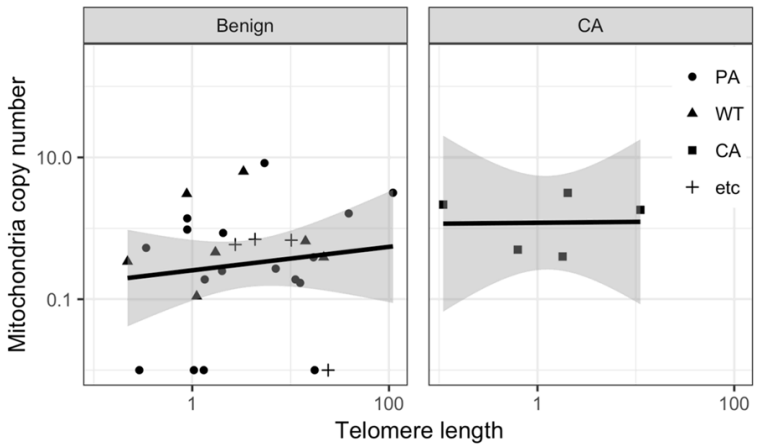

Figure 3. Scatter plot of mtDNA copy number with TL according to histologic group of the salivary gland tumors. The black continuous line and a gray area denote linear regression line and residual value by Spearman correlation analysis. PA (closed circle), pleomorphic adenoma; WT (closed triangle), Warthin's tumor; CA (closed rectangle), carcinoma; mtCN, mtDNA copy number.

characteristics for various histologic types. By dividing three groups such as PA, WT, and carcinoma, we expected to reveal differences of molecular characteristics according to their known malignant potential.

The mechanism of malignant transformation is still unclear, and the accumulation of genetic instabilities is one of the possible hypotheses. In a study with CXPA, they found that an oncogenic rearrangement of PLAG1 (pleomorphic adenoma gene 1) and HMGA2 (high mobility group A2) persisted [33]. In addition, the immunohistochemical staining of CXPA showed high expression of Ki-67, p53, and HER-2 protein. For those reasons, several studies tried to reveal the relevance of those proteins in the malignant transformation of PA [34, 35].

PI3K/Akt pathway has been well known to represent an important regulator of cellular proliferation, survival, and motility, and through many studies, the pathway might be involved in malignant transformation of many different cancers. The relationship of salivary gland cancer and PIK3CA mutation has been already studied, and especially in salivary duct carcinoma, PIK3CA mutations frequently occurred [24, 25]. In our study, there was no significant difference in PIK3CA amplification among the 3 groups. WT showed slightly decreased PIK3CA amplification with no statistical significance. Our malignancy group was heterogeneous, but another study revealed that PIK3CA amplification is present in only 29% of adenocystic carcinoma [36]. Considering the aforementioned studies and our study with a small number of malignant salivary tumors, more cases of malignant tumors should be recruited, and histologic types of malignancies should be studied separately to clarify the role of PIK3CA amplification in salivary gland tumors.

Telomeric DNA undergoes progressive shortening with each cell division, and consequently, cells manifest apoptosis. When apoptosis is blocked by disrupting regulating pathways such as p53 or Rb pathways, dysfunctional telomeres result, and genomic instability is induced. Thus, the risk of activation of telomere maintenance mechanisms and oncogenesis could be increased [37]. Our results of TL showed no differences between the three groups. This result is inconsistent with other previous studies which compared PA and CXPA [38]. This result might reflect the small size and heterogeneity of the carcinomas. Interestingly, there were no differences in TL between WT and PA.

There are recent reports that the shortening of TL and mitochondrial dysfunction co-contribute to aging [39, 40] and degenerative disease [41]. We previously reported that genetic change in mtDNA could be associated with the tubular adenoma-carcinoma sequence in colorectal carcinoma [42]. We also reported that TL was associated with mtCN in normal tissues and colorectal cancer, but not in the precancerous lesions of colorectal cancer [22]. Here, we analyzed the association of TL and mtCN in the benign and malignant tumor of the salivary gland; however, no correlation was found.

Patient age did not affect the cancer-related gene profile, but the size of PA showed a significant relationship with TL. The incidence of malignant transformation has been known to increase with the length of history of PA, from 1.5% at five years to 10% after 15 years [8]. Based on the dividing properties of tumor cells,

the size of the tumor reflects the length of history of PA, during which the possibility of malignant transformation to CXPA increases. Therefore, the correlation between tumor size and TL in PA suggests that TL may be a marker of malignant transformation of PA.

Several studies have revealed an association with genetic profile and salivary malignancy. The novelty of our study is that we compared three tumor types that have different malignant potential: WT with very low malignant potential, PA with low-moderate malignant potential, and carcinoma. We tried to show differences of cancer-related gene profiles according to known malignant potential of benign salivary gland tumor. However, we had a small size in the carcinoma group, and it was heterogeneous. Therefore, a further large-scale study is needed.

In conclusion, PA and WT are the most common benign tumors of the parotid gland, and they are known to have malignant transformation potential. We tried to show differences in cancer-related gene profiles such as PIK3CA amplification, TL, and mtCN. However, there were no differences between WT, PA, and carcinoma. The size of PA showed a significant relationship with TL. Although we have not found any significant results for the molecular profile of salivary gland tumors, our study can be a basis of further studies on other oncogenes in salivary gland tumors.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Heejin Kim, Department of Otorhinolaryngology-Head and Neck Surgery, Hallym University Dongtan Sacred Heart Hospital, Keunjaebonggil 7, Seokwoodong, Hwaseong, Kyeonggido, South Korea. Tel: +82-31-8086-2670; Fax: +82-31-8086-2029; E-mail: heejin5020@hanmail.net

References

[1] Eveson JW, Auclair PL, Gnepp DR. Tumours of the salivary glands: Chapter 5. World Health Organization Classification of Tumours: Pathology and Genetics. Lyon, France: IARC Press; 2005. pp. 209-281.

[2] Seethala RR and Stenman G. Update from the 4th edition of the World Health Organization classification of head and neck tumours: tumors of the salivary gland. Head Neck Pathol 2017; 11: 55-67.

[3] Jung KW, Won YJ, Oh CM, Kong HJ, Lee DH, Lee KH. Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2014. Cancer Res Treat 2017; 49: 292-305.

[4] Takahama Junior A, Almeida OP, Kowalski LP. Parotid neoplasms: analysis of 600 patients attended at a single institution. Braz J Otorhinolaryngol 2009; 75: 497-501.

[5] Al-Khateeb TH, Ababneh KT. Salivary tumors in North Jordanians: a descriptive study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007; 103: e53-e9.

[6] Lee YM, Choi HJ, Kim JW, Kim JH. Parotid gland tumors in a Korean population. J Craniofac Surg 2012; 23: e205-9.

[7] Boukheris H, Curtis RE, Land CE, Dores GM. Incidence of carcinoma of the major salivary glands according to the WHO classification, 1992 to 2006: a population-based study in the United States. Cancer Epidemiol Biomarkers Prev 2009; 18: 2899-906.

[8] Gnepp DR. Malignant mixed tumors of the salivary glands; a review. Pathol Annu 1993; 28: 279-328.

[9] Antony J, Gopalan V, Smith RA, Lam AK. Carcinoma ex pleomorphic adenoma: a comprehensive review of clinical, pathological and molecular data. Head Neck Pathol 2012; 6: 1-9.

[10] Andreasen S, Therkildsen MH, Bjørndal K, Homøe P. Pleomorphic adenoma of the parotid gland 1985-2010: a Danish nationwide study of incidence, recurrence rate, and malignant transformation. Head Neck 2016; 38 Suppl 1: E1364-9.

[11] Valstar MH, de Ridder M, van den Broek EC, Stuiver MM, van Dijk BAC, van Velthuysen MLF, Balm AJM, Smeele LE. Salivary gland pleomorphic adenoma in the Netherlands: a nationwide observational study of primary tumor incidence, malignant transformation, recurrence, and risk factors for recurrence. Oral Oncol 2017; 66: 93-9.

[12] Bell D, Luna MA. Warthin adenocarcinoma: analysis of 2 cases of a distinct salivary neoplasm. Ann Diagn Pathol 2009; 13: 201-7.

[13] Horn S, Figl A, Rachakonda PS, Fischer C, Sucker A, Gast A, Kadel S, Moll I, Nagore E, Hemminki K, Schadendorf D, Kumar P. TERT promoter mutations in familial and sporadic melanoma. Science 2013; 339: 959-61.

[14] Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA. Highly recurrent TERT promoter

mutations in human melanoma. Science 2013; 339: 957-9.
[15] Lee H, Jin JD, La BM, Park WJ, Choi IJ, Lee JH. TERT promoter mutation, telomere length, and TERT expression in gastric cancer. Int J Clin Exp Pathol 2016; 9: 1758-63.
[16] Qu Y, Shi L, Wang D, Zhang B, Yang Q, Ji M, Shi B, Hou P. Low frequency of TERT promoter mutations in a large cohort of gallbladder and gastric cancers. Int J Cancer 2014; 134: 2993-4.
[17] Liu T, Wang N, Cao J, Sofiadis A, Dinets A, Zedenius J, Larsson C, Xu D. The age- and shorter telomere-dependent TERT promoter mutation in follicular thyroid cell-derived carcinomas. Oncogene 2014; 33: 4978-84.
[18] Borah S, Xi L, Zaug AJ, Powell NM, Dancik GM, Cohen SB, Costello JC, Theodorescu D, Cech TR. Cancer. TERT promoter mutations and telomerase reactivation in urothelial cancer. Science 2015; 347: 1006-10.
[19] Kim H, Ahn D, Sohn JH, Kim YH, Lee JH, Lee H. TERT promoter mutation and telomere length in salivary gland tumors. Pathol Oncol Res 2018; 24: 697-8.
[20] Mithani SK, Shao C, Tan M, Smith IM, Califano JA, El-Naggar AK, Ha PK. Mitochondrial mutations in adenoid cystic carcinoma of the salivary glands. PLoS One 2009; 4: e8493.
[21] Yu M. Generation, function and diagnostic value of mitochondrial DNA copy number alterations in human cancers. Life Sci 2011; 89: 65-71.
[22] Lee H, Cho JH, Park WJ, Jung SJ, Choi IJ, Lee JH. Loss of the association between telomere length and mitochondrial DNA copy number contribute to colorectal carcinogenesis. Pathol Oncol Res 2018; 24: 323-8.
[23] Jung SJ, Cho JH, Park WJ, Heo YR, Lee JH. Telomere length is correlated with mitochondrial DNA copy number in intestinal, but not diffuse, gastric cancer. Oncol Lett 2017; 14: 925-9.
[24] Griffith CC, Seethala RR, Luvison A, Miller M, Chiosea SI. PIK3CA mutations and PTEN loss in salivary duct carcinomas. Am J Surg Pathol 2013; 37: 1201-7.
[25] Qiu W, Tong GX, Turk AT, Close LG, Caruana SM, Su GH. Oncogenic PIK3CA mutation and dysregulation in human salivary duct carcinoma. Biomed Res Int 2014; 2014: 810487.
[26] Shalmon B, Drendel M, Wolf M, Hirshberg A, Cohen Y. Mutational analysis of PI3K/AKT and RAS/RAF pathway activation in malignant salivary gland tumours with a new mutation of PIK3CA. Int J Oral Maxillofac Surg 2016; 45: 721-5.
[27] Yuan TL, Cantley LC. PI3K pathway alterations in cancer: variations on a theme. Oncogene 2008; 27: 5497-510.
[28] Lee H, Hwang IS, Choi IJ, Kang YN, Park KU, Lee JH. Are PIK3CA mutation and amplification associated with clinicopathological characteristics of gastric cancer? Asian Pac J Cancer Prev 2015; 16: 4493-6.
[29] Team RC. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria: 2016.
[30] Wickham H. ggplot2: elegant graphics for data analysis. Springer-Verlag: New York, NY; 2009.
[31] Seifert G, Brocheriou C, Cardesa A, Eveson JW. WHO international histological classification of tumours tentative histological classification of salivary gland tumours. Pathol Res Pract 1990; 186: 555-81.
[32] Nouraei SA, Hope KL, Kelly CG, McLean NR, Soames JV. Carcinoma ex benign pleomorphic adenoma of the parotid gland. Plast Reconstr Surg 2005; 116: 1206-13.
[33] Bahrami A, Dalton JD, Shivakumar B, Krane JF. PLAG1 alteration in carcinoma ex pleomorphic adenoma: immunohistochemical and fluorescence in situ hybridization studies of 22 cases. Head Neck Pathol 2012; 6: 328-35.
[34] Hashimoto K, Yamamoto H, Shiratsuchi H, Nakashima T, Tamiya S, Nishiyama K, Higaki Y, Komune S, Tsuneyoshi M, Oda Y. HER-2/neu gene amplification in carcinoma ex pleomorphic adenoma in relation to progression and prognosis: a chromogenic in-situ hybridization study. Histopathology 2012; 60: E131-42.
[35] Katori H, Nozawa A, Tsukuda M. Increased expression of cyclooxygenase-2 and Ki-67 are associated with malignant transformation of pleomorphic adenoma. Auris Nasus Larynx 2007; 34: 79-84.
[36] Sequeiros-Santiago G, García-Carracedo D, Fresno MF, Suarez C, Rodrigo JP, Gonzalez MV. Oncogene amplification pattern in adenoid cystic carcinoma of the salivary glands. Oncol Rep 2009; 21: 1215-22.
[37] Shay JW, Wright WE. Role of telomeres and telomerase in cancer. In: Seminars in cancer biology, Vol. 21, No. 6. Academic Press; 2011. pp. 349-53.
[38] Nikolic N, Anicic B, Carkic J, Simonovic J, Toljic B, Tanic N, Tepavcevic Z, Vukadinovic M, Konstantinovic VS, Milasin J. High frequency of p16 and p14 promoter hypermethylation and marked telomere instability in salivary gland tumors. Arch Oral Biol 2015; 60: 1662-6.
[39] Sahin E, Colla S, Liesa M, Moslehi J, Müller FL, Guo M, Cooper M, Kotton D, Fabian AJ, Walkey C, Maser RS, Tonon G, Foerster F, Xiong R, Wang YA, Shukla SA, Jaskelioff M, Martin ES, Heffernan TP, Protopopov A, Ivanova E, Mahoney JE, Kost-Alimova M, Perry SR, Bronson R, Liao R, Mulligan R, Shirihai OS, Chin L, DePinho RA. Telomere dysfunction induces meta-

bolic and mitochondrial compromise. Nature 2011; 470: 359-65.
[40] Sahin E, DePinho RA. Axis of ageing: telomeres, p53 and mitochondria. Nat Rev Mol Cell Biol 2012; 13: 397-404.
[41] Dechsupa S, Limthongkul W, Yingsakmongkol W, Singhatanadgige W, Honsawek S. Relative telomere length and mitochondria DNA copy number in ligamentum flavum of lumbar stenosis patients. J Orthop Translat 2016; 7: 132.
[42] Lee JH, Hwang I, Kang YN, Choi IJ, Kim DK. Genetic characteristics of mitochondrial DNA was associated with colorectal carcinogenesis and its prognosis. PLoS One 2015; 10: e0118612.