ORIGINAL ARTICLE

UDC: 616.316-006-037
DOI: https://doi.org/10.2298/VSP201026130D

Expression of apoptotic protease-activating factor-1 in adenoid cystic carcinoma of the salivary glands and its clinicopathological relevance

Ekspresija faktora-1 aktivacije proteaza apoptoze u adenoidnom cističnom karcinomu pljuvačnih žlezda i njegov kliničkopatološki značaj

Branko Dožić*, Boban Aničić†, Vladimir Sinobad†, Nikola Miković†, Srdjan Milanović‡, Ivan Dožić§

University of Belgrade, Faculty of Dental Medicine, *Department of Pathology, †Clinic of Maxillofacial Surgery, §Department of Biochemistry, Belgrade, Serbia; ‡Institute for Oncology and Radiology of Serbia, Belgrade, Serbia

Abstract

Background/Aim. Apoptotic protease-activating factor-1 (Apaf-1) is a key molecule in the intrinsic or mitochondrial pathway of apoptosis. Some pathological conditions, such as cancer, stroke, and neurodegenerative diseases, are the result of dysregulation in the intrinsic apoptosis pathway. The aim of this study was to analyze the immunohistochemical expression of Apaf-1 in adenoid cystic carcinoma (ACC) cells of the salivary glands and its correlation with clinicopathological parameters of patients (gender, age, localization, histological type, and overall survival). **Methods.** Formalin-fixed, paraffin-embedded tissues of ACC of the salivary glands from 50 male and female patients with an average age of 58 years, were used for the study. We used the technique of tissue microarray (TMA blocks). Sections from the TMA mold, 5 μm thick, were stained with the streptavidin-biotin immunohistochemical technique using primary antibodies specific for Apaf-1 (Leica Biosystems, Newcastle, UK). Stained tissue sections were analyzed by the light microscope (Olympus type BH-2). Based on the data collected, the database was created in SPSS software v. 22.0 (SPSS Inc., Chicago, ILL, USA), which was used for further statistical analysis. The statistical data analysis included methods of descriptive and analytical (inferential) statistics. **Results.** The results of the immunohistochemical analysis of Apaf-1 expression in the samples of patients with ACC of the salivary glands were compared with the clinicopathological parameters of these patients. The immunohistochemical expression of Apaf-1 showed no statistical significance with regard to the patients' gender ($p = 0.552$), age ($p = 0.106$), histological tumor type ($p = 0.654$), and localization of ACC in the salivary glands ($p = 0.486$). There was no statistically significant correlation observed between the overall survival of ACC patients and Apaf-1 expression in tumor cells ($p = 0.340$, Log-Rank test). **Conclusion.** With regard to ACC, Apaf-1 expression is not in correlation with clinicopathological parameters (gender, age, localization, histological tumor type, outcome of the disease, and overall survival). Therefore, we believe Apaf-1 cannot be regarded as an independent prognostic factor for course and outcome of ACC of the salivary glands.

Key words:
sex; apoptosis regulatory proteins; age factor; immunohistochemistry; apoptotic protease-activating factor; carcinoma, adenoid, cystic; survival.

Apstrakt

Uvod/Cilj. Faktor 1 aktivacije proteaza apoptoze (Apaf-1) je ključni molekul u unutrašnjem ili mitohondrijskom putu apoptoze. Različita patološka stanja, kao na primer maligniteti, neurodegenerativne bolesti, moždani udar, povezuju se sa poremećajem unutrašnjeg puta apoptoze. Cilj rada bio je da se analizira imunohistohemijska ekspresija Apaf-1 u ćelijama adenoidnog cističnog karcinoma (ACC) pljuvačnih žlezda i njegova korelacija sa kliničko-patološkim parametrima obolelih (pol, starost, lokalizacija, histološki tip i ukupno preživljavanje). **Metode**. U studiji su korišćeni parafinski uzorci ACC pljuvačnih žlezda 50 obolelih muškaraca i žena, prosečne starosti 58 godina. Korišćena je tehnika mikrotkivnog niza (*tissue microarrays* – TMA *block*). Mikrotkivni preseci, debljine 5 μm, bojeni su streptavidin-biotin imunohistohemijskom tehnikom primenom primarnog antitela specifičnog za Apaf-1 (Leica Biosystems, Newcastle, UK). Preparati su analizirani svetlosnim mikroskopom (Olympwus type BH-2). Na osnovu prikupljenih podataka kreirana je baza podataka u SPSS softveru 22.0 (SPSS Inc., Chicago, ILL, USA) koji je korišćen za dalju statističku analizu. Statistička analiza podataka obuhvatala je metodu deskriptivne i analitičke

Correspondence to: Ivan Dožić, University of Belgrade, Faculty of Dental Medicine, Department of Biochemistry, Dr Subotića br. 1, 11 000 Belgrade, Serbia. E-mail: ivan.dozic@stomf.bg.ac.rs, drivandozic@gmail.com

(inferencijalne) statistike. **Rezultati**. Rezultati imunohistohemijske analize ekspresije Apaf-1 u uzorcima bolesnika sa ACC pljuvačnih žlezda upoređeni su sa njihovim kliničko-patološkim parametrima. Ekspresija Apaf-1 nije pokazala statističku značajnost u odnosu na pol bolesnika (p = 0,552), starost (p = 0,106), histološki tip tumora (p = 0,654) i lokalizaciju ACC u pljuvačnim žlezdama (p = 0,486). Nije primećena statistički značajna korelacija između ekspresije Apaf-1 i preživljavanja bolesnika sa ACC (p = 0,340, Log-Rank test). **Zaključak**. U odnosu na ACC, ekspresija Apaf-1 nije u korelaciji sa kliničko-patološkim parametrima bolesnika (pol, starost, lokalizacija, histološki tip i ukupno preživljavanje). Zato verujemo da se Apaf-1 ne može smatrati nezavisnim prognostičkim faktorom za tok i ishod ACC pljuvačnih žlezda.

Ključne reči:
pol; apoptoza, regulatorni proteini; životno doba, faktor; imunohistohemija; faktor-1, proteaza-aktivirajući, apoptotički; karcinom, adenoidni cistični; preživljavanje.

Introduction

According to the latest WHO histological classification [1], salivary gland carcinomas encompass over 20 histological types and comprise 8.5% of all head and neck carcinomas [2]. Being one of the most frequent malignant tumors affecting the salivary glands, adenoid cystic carcinoma (ACC) comprises 3–5% of all head and neck malignancies. While ACC may develop in all salivary glands, it is mainly located in the minor salivary glands, usually in the palate. Slow growth, extensive local invasion, perineural spread, and the late development of distant metastases are characteristic of ACC [3–5]. Microscopically, ACC may show a cribriform, tubular, or solid histopathological pattern, even though all three patterns are present in most cases, with one pattern being dominant [6, 7].

The primary goal of treating ACC patients is to control the disease locally while maintaining normal functioning and preventing distant metastases. Therefore, surgical resection of the tumor is the therapy of choice in most cases. However, given the nature of the lesion, adjuvant therapy is used besides surgical resection. The role of postoperative radiotherapy is still considered controversial due to divergent reports concerning its effects on ACC patients. Moreover, the role of chemotherapy in ACC patients has not been adequately defined yet [8].

In recent years, there has been extensive research into new biological markers in order to foresee the efficacy of these therapies with more confidence. Nowadays, there is a particular interest in the molecules involved in programmed cell death – apoptosis [9, 10]. There are two apoptotic pathways: extrinsic (receptor) and intrinsic (mitochondrial). Apoptotic protease-activating factor-1 (Apaf-1) has an important role in the intrinsic apoptotic pathway. Apaf-1 is a multidomain protein (139 kDa) that contains the amino-terminal domain, the central oligomeric nucleotide-binding domain, and the carboxy-terminal domain. The Apaf-1 protein binds cytochrome c released from mitochondria and initiates conformational changes and the formation of a multiprotein complex – apoptosome, comprised of cytochrome c, Apaf-1, and procaspase-9. This complex proteolytically activates the caspase-9 enzyme, whose role is to activate effector caspases (caspase-3 and -7) in cascade reactions, which then initiate the degradation phase of apoptosis [11–14, 15].

The evaluation of immunohistochemical expression of Apaf-1 in relation to clinicopathological parameters of patients has been conducted on various types of human tumors. Some authors have indicated that lower expression of Apaf-1 can represent the trigger for the malignant transformation of cells and can also be the cause of chemotherapy resistance [16–18].

Therefore, the aim of this study was to analyze the immunohistochemical expression of Apaf-1 in ACC tumor cells of the salivary glands and its correlation with clinicopathological parameters of patients.

Methods

This study included 50 patients diagnosed with ACC of the salivary glands. From the paraffin blocks, from each case, three cylinders of tissue were taken from the tumor area with a donor block with a diameter of 1.2 mm and placed in the three recipient paraffin blocks (TMA blocks) every 62 cores. Control (human myocardium tissue) is also included in the TMA blocks.

Five-micrometer cut sections from TMA blocks were deparaffinized, rehydrated, placed in 3% H_2O_2 for 10 min to block endogenous peroxidase activity, and washed with tap water. Then, they were processed with 0.01 citrate buffer (pH 6.0) and treated in a microwave oven for 20 min at 600 W, and placed in a bath of tap water for 20 min, then in distilled water and TBS buffer (pH 7.6) for 5 min, and placed in diluted normal serum for 10 min. Afterward, the tissue sections were incubated for 1 h with the following rabbit monoclonal primary antibody Apaf-1, Product Code: NCL-APAF1, dilution 1:40, Leica Biosystems, Newcastle, UK.

Streptavidin-biotin method using DAKO's LSAB+ kit (DAKO, Denmark) was applied, with diaminobenzidine (DAB) as the chromogen solution and Mayer's hematoxylin for the counterstain.

All immunostained sections were independently evaluated by two pathologists. The results of immunohistochemical staining Apaf-1 were scored by a semiquantitative technique into 3 grades according to the intensity of the staining: 1+ (weak or negative), 2+ (moderate), and 3+ (strong).

Statistical analysis

Statistical analyses were performed using SPSS software v. 22.0 (SPSS Inc., Chicago, ILL, USA). Descriptive

data for all groups and variables were expressed as mean ± standard deviation (SD) for continuous variables or percent of a group for discrete variables (categorical data). Categorical data were analyzed using the Pearson's χ^2 test. Normal distribution was tested using the Kolmogorov-Smirnov test, and the normal distribution of continuous data was tested with a one-way ANOVA test. Kappa coefficient of agreement was used for the evaluation of correspondence between two pathologists. Overall survival rates were calculated from the day of diagnosis by the Kaplan-Meier method, and differences were evaluated by the Log-Rank test. All reported *p*-values were two-sided. Differences were considered significant when the *p*-value was < 0.05.

Results

This study used the streptavidin-biotin immunohistochemical method in order to investigate the immunohistochemical expression of Apaf-1 in ACC cells of the salivary glands in relation to clinicopathological parameters of the patients. The evaluation was performed on biopsy samples of 50 patients (19 male and 31 female), with a mean age of 58 years (range 28–78 years). Statistical analysis showed a high degree of interobserver concordance between the two pathologists ($\kappa = 0.817$).

In ACC cells, Apaf-1 was expressed in their cytoplasm. In most cases (42%), the intensity of Apaf-1 expression was strong (3+) (Figure 1a). Moderate expression (2+) was observed in 15 (30%) samples (Figure 1b), while 14 (28%) samples showed Apaf-1 expression graded as 1+ (Figure 1c). The differences in the intensity of Apaf-1 expression in ACC cells in the salivary glands were not found statistically significant ($p = 0.423$) (Table 1).

The results of the immunohistochemical analysis of Apaf-1 expression in the samples of patients with ACC of the salivary glands were compared with the clinicopathologi-

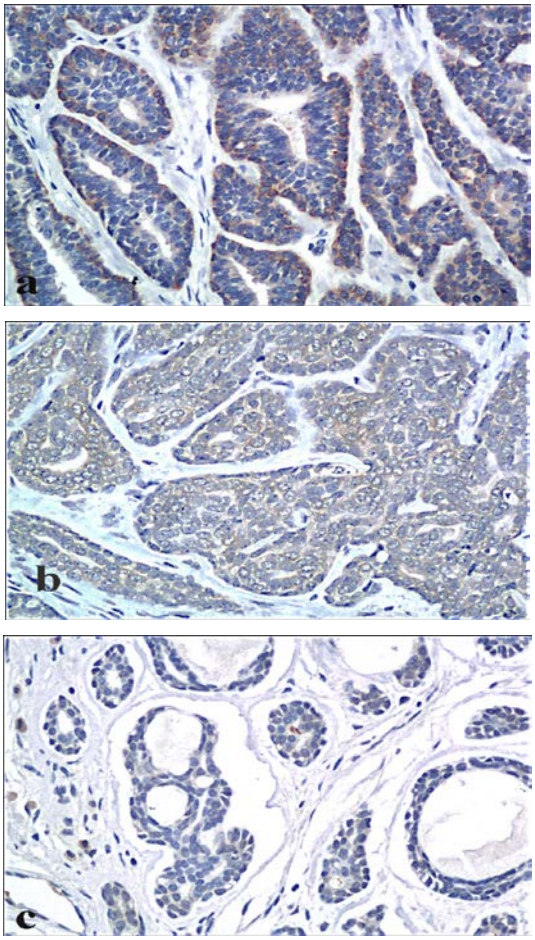

Fig. 1 – Streptovidin-biotin immunohistochemical staining of adenoid cystic carcinoma (ACC) tumor cells in the salivary glands: a) strong (×400), b) moderate (×200), and c) weak (×200) intensity of apoptotic protease-activating factor-1 (Apaf-1) expression.

Table 1

Apoptotic protease-activating factor-1 (Apaf-1) expression in adenoid cystic carcinoma (ACC) of the salivary glands and its correlation with patient's clinicopathological parameters

Patient's characteristics	Apaf-1 expression			Significance (*p*-value)
	+	++	+++	
ACC, n (%)	14 (28.0)	15 (30.0)	21 (42.0)	[a]0.423
Gender, n (%)				
male	7 (36.8%)	5 (26.4%)	7 (36.8%)	[a]0.552
female	7 (22.6%)	10 (32.3%)	14 (45.2%)	
Age (years), mean ± SD	52.50 ± 15.42	57.80 ± 12.5	61.81 ± 10.23	[b]0.106
(median; min-max)	(56.5; 29–78)	(56; 39–78)	(61; 41–75)	
Localization, n (%)				
parotid	1 (25.0)	1 (25.0)	2 50.0)	
submandibular	1 (11.2)	4 (44.4)	4 (44.4)	
hard palate	8 (27.6)	7 (24.1)	14 (48.3)	[a]0.486
minor salivary gland	4 (50.0)	3 (37.5)	1 (12.5)	
Histological type, n (%)				
cribriform	2 (14.3)	5 (35.7)	7 (50.0)	
tubular	6 (33.3)	4 (22.2)	8 (44.4)	[a]0.654
solid	6 (33.3)	6 (33.3)	6 (33.3)	
Outcome, n (%)				
surviving	6 (31.6)	7 (36.8)	6 (31.6)	[a]0.494
deceased	8 (25.8)	8 (25.8)	15 (48.4)	

n – number of patients; SD – standard deviation.

[a]χ^2-test; [b]One-way ANOVA.

cal parameters of these patients (Table 1). The immunohistochemical expression of Apaf-1 showed no statistical significance with regard to the patients' gender (p = 0.552) and age (p = 0.106). In male patients, the grades 3+ and 1+ were equally represented, with 5 patients showing moderate (2+) Apaf-1 expression. The intensity of Apaf-1 expression in female patients was most frequently graded as 3+ (14/31). There were 10 female patients showing moderate (2+) Apaf-1 expression and 7 female patients with weak (1+) Apaf-1 expression.

Concerning the age of the patients, Apaf-1 expression of 1+ or 2+ was observed in the patients whose mean age was 56 years, whereas Apaf-1 expression graded as 3+ was observed in the patients whose mean age was 61 years (Table 1).

Regarding the localization of ACC in the salivary glands, the intensity of Apaf-1 expression was not found statistically significant (p = 0.486) (Table 1). ACC located in the minor salivary glands of the hard palate demonstrated strong Apaf-1 expression (3+) in 48% of cases. There was an approximate number of cases with moderate (24.1%) and weak (27.6%) intensities of Apaf-1 expression. A similar intensity of Apaf-1 expression was observed in ACC of the parotid glands. As opposed to these findings, the tumors located in the submandibular salivary gland showed the same percentage (44.4%) of strong and moderate intensities of Apaf-1 expression, while in 11.2% of cases, Apaf-1 expression was graded as 1+.

No statistically significant correlation was detected between the histological tumor type and Apaf-1 expression (p = 0.654) (Table 1). Apaf-1 expression in tumors with the cribriform pattern was most frequently graded as 3+ (50%). In 35.7% of cases, the intensity of Apaf-1 expression was graded as moderate, and in 14.3% of cases, it was graded as 1+. The percentage of Apaf-1 expression graded as 3+ was somewhat lower in ACC with the tubular pattern (44.4%), while Apaf-1 expression graded as 1+ was present to a higher extent when compared to tumors with the cribriform pattern. When it comes to solid tumors, the three different grades of Apaf-1 expression were equally distributed.

The present study also investigated the significance of evaluating Apaf-1 expression in relation to the outcome of the disease (Table 1). The percentages of different Apaf-1 expression grades were quite similar in patients who were alive at the end of this investigation. Strong immunopositivity (3+) was observed in tumor cells of approximately 50% of deceased patients. The evaluation of Apaf-1 expression was not found statistically significant in relation to surviving and deceased patients (p = 0.494).

There was no statistically significant correlation observed between the overall survival of ACC patients and Apaf-1 expression in tumor cells (p = 0.340, Log-Rank test) (Table 2, Figure 2).

Table 2

Apoptotic protease-activating factor-1 (Apaf-1) expression in adenoid cystic carcinoma (ACC) of the salivary glands in relation to the 13-year survival period

Grade of Apaf-1 expression	Follow-up period (years)								Significance (p-value)
	1	3	5	7	9	10	11	13	
+	92.9	78.6	78.6	71.4	63.5	50.8	16.9	16.9	
++	93.3	93.3	80.0	53.3	53.3	53.3	53.3	26.7	#0.340
+++	90.5	66.7	47.6	47.6	33.3	33.3	22.2	22.2	

All values are expressed as percentages (%) of the patients.
#Log-Rank test.

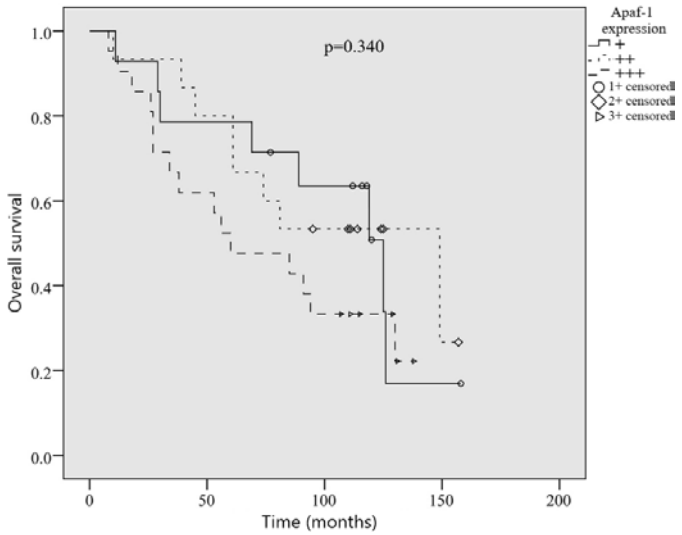

Fig. 2 – Overall survival of patients with adenoma cystic carcinoma (ACC) in relation to apoptotic protease-activating factor-1 (Apaf-1) expression.

The mean survival period of patients with Apaf-1 expression of 1+ was 125 months. In these patients, the 5-year survival rate was 78.6%, the 10-year survival rate was 50.8%, and the 13-year survival rate was 16.9%. The patients with Apaf-1 expression graded as 2+ had the longest mean survival period of 149 months. Their 5-year survival rate was 80%, while their 10- and 13-year survival rates were 53.3% and 26.7%, respectively. The shortest mean survival period of 60 months was detected in the patients with Apaf-1 expression of 3+. Their 5-year survival rate was 65%, whereas their 10- and 13-year survival rates were 45.8% and 22.9%, respectively.

Discussion

Salivary gland carcinoma is characterized by a disbalance of regulatory mechanisms of different cellular pathways, including apoptosis. Apaf-1 is a key effector molecule of the intrinsic apoptotic pathway, and it has been investigated in various human tumors [15–18].

Our findings suggest that ACC cells in the salivary glands show Apaf-1 immunopositivity in most cases. Two independent pathologists evaluated the intensity of cytoplasmic expression in the sections from TMA blocks with a high degree of interobserver concordance. Strong Apaf-1 expression was detected in 42% of cases, while moderate Apaf-1 expression was observed in 30% of cases. Weak or negative Apaf-1 expression was found in 28% of patients.

The evaluation of immunohistochemical expression of the antibody typical of Apaf-1 has been conducted on patients with malignant melanomas. The level of Apaf-1 cytoplasmic expression was determined in the samples from TMA blocks. As opposed to our findings, in 36 patients out of 70 (51.4%) diagnosed with melanoma, Apaf-1 expression was graded as weak. Moderate expression was observed in 21 (30%) patients, while strong expression was found in 7 (10%) patients. Six melanoma patients (8.6%) did not show immunopositivity for this antibody [15]. Significantly reduced expression of Apaf-1 molecules has been observed in colon adenocarcinoma. Out of 529 patients with colon adenocarcinoma, only 129 (22%) reacted positively to the antibody typical of Apaf-1 [17]. Since Apaf-1 expression was graded as strong in 76.9% of cases, Apaf-1 has been characterized as a highly immunopositive marker, which is in line with our findings observed in nevus cells. The loss of Apaf-1 expression can be the trigger for initiating a malignant transformation of melanocytes. Furthermore, it is argued that reduced Apaf-1 expression in melanoma cells, which have demonstrated weak Apaf-1 expression in most cases, can be the cause of chemotherapy resistance [15]. Given the fact that adenomas also showed 100% positivity for Apaf-1 antibody, the authors believe that reduced Apaf-1 expression in adenocarcinoma is in correlation with the malignant alteration of adenoma [17].

Cytoplasmic positivity for Apaf-1 antibody was observed in cervix carcinoma, which is in accordance with our findings. Out of 86 patients diagnosed with cervix carcinoma, 42% showed strong immunopositivity to Apaf-1. Moderate expression was observed in 34% and weak or negative in 24% of patients [16].

The ability of tumor cells to resist apoptotic signals can affect the aggressiveness and, therefore, the prognosis. Several studies have reported the results of the expression, *i.e.*, the activity, of the factor of apoptotic signaling, which affects the apoptotic resistance of tumor cells or cell lines in the *in vitro* conditions or animal models. However, the correlation of these results with clinicopathological parameters and malignancy prognosis is particularly significant [19–21]. The correlation between Apaf-1 expression and clinicopathological parameters of ACC patients was not found statistically significant in our study. Apaf-1 expression was most frequently graded as 1+ or 3+ in 19 male patients. Apaf-1 expression of 3+ was observed in 14 out of 31 female patients. The mean age of the patients with expressions of 1+ and 2+ was 56 years, while in patients with expressions of 3+, it was 61 years. The tumors affecting the minor salivary glands of the hard palate, which was the most common localization in the present study, showed Apaf-1 expression of 3+ in approximately 50% of cases. The correlation between the histological type and Apaf-1 expression was not found statistically significant either. Apaf-1 expression detected in cribriform and tubular tumors was mainly graded as 3+. An equal distribution of the three different grades of Apaf-1 expression was observed in the solid variant of ACC, which is commonly associated with a less favorable prognosis and the formation of distant metastases.

Our findings are in line with the findings reported about melanomas since Apaf-1 expression was not found statistically significant concerning gender, age, histological subtype, and localization [15].

As opposed to our findings, reduced Apaf-1 expression in colorectal carcinoma was in correlation with poor prognostic factors such as depth of invasion, formation of metastases in regional lymph nodes, and histological grading [18]. In patients diagnosed with cervix carcinoma, there was a significant correlation between Apaf-1 expression and nodal status. The patients with strong or moderate Apaf-1 expression had a significantly lower number of metastases in the lymph nodes at the time of surgical intervention when compared to the patients with weak or negative expression [16].

According to the latest reports, the survival rates following surgical removal of ACC is 70% after 5 years and 60% after 10 years [3, 4].

Furthermore, Apaf-1 expression was not statistically significant to the overall survival of ACC patients. In summary, the number of surviving patients with Apaf-1 expression of 1+ and 2+ is approximately the same at the 5- and 10-year follow-ups. A lower survival rate was observed in the patients with Apaf-1 expression of 3+. In these patients, the survival rate was 47.6% after 5 years and 33.3% after 10 years. The mean survival period of ACC patients was the longest in the patients with expression graded as 2+ (149 months), while the shortest mean survival period was observed in the patients with expression graded as 2+ (60 months). Concerning the surviving and deceased patients, Apaf-1 was not found statistically significant. The patients who were alive at the end of the follow-up period had almost equal percentages of different Apaf-1 ex-

pression grades. Apaf-1 expression of 3+ was more common in the deceased patients, *i.e.*, in 15 out of 31 deceased patients.

Our findings are in line with the findings obtained in the studies investigating Apaf-1 and the survival of patients with melanoma [15] and colorectal carcinoma [18]. The 5-year survival rate of patients diagnosed with colorectal carcinoma who had negative Apaf-1 expression was 67.7%, and their 10-year survival rate was 62.3%. The five-year survival rate of patients with positive Apaf-1 expression was 72.7%, and their 10-year survival rate was 67.1%. These findings are in accordance with the findings obtained by Zlobec et al. [17], who investigated Apaf-1 expression in a large series of 1,420 colorectal carcinomas.

Conclusion

With regard to ACC, Apaf-1 expression is not in correlation with clinicopathological parameters of patients (gender, age, localization, histological tumor type, outcome of the disease, and overall survival). Therefore, we believe Apaf-1 expression cannot be regarded as an independent prognostic factor for course and outcome of ACC.

Conflict of interest

The authors declare no conflict of interest. This study received no funding.

R E F E R E N C E S

1. *Rossi DE, Baloch Z, Pusztaszeri M, Faquin CW*. The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC): an ASC-IAC–sponsored system for reporting salivary gland fine-needle aspiration. J Am Soc Cytopathol 2018; 7(3): 111–8.
2. *Guzzo M, Locati LD, Prott FJ, Gatta G, McGurk M, Licitra L*. Major and minor salivary gland tumors. Crit Rev Oncol Hematol 2010; 74(2): 134–48.
3. *Ishida E, Ogawa T, Rokugo M, Ishikawa T, Wakamori S, Ohkoshi A,* et al. Management of adenoid cystic carcinoma of the head and neck: a single-institute study with over 25-year follow-up. Head Face Med 2020; 16(1): 14.
4. *Xu L, Li L, Zhang J, Cai W, Zhao S, Liu S*. Accumulated cytotoxicity of CDK inhibitor dinaciclib with first-line chemotherapy drugs in salivary adenoid cystic carcinoma cells. Odontology 2020; 108(2): 300–11.
5. *Shizhi H, Pingdong L, Qi Z, Lizhen H, Zhenkun Y, Zhigang H,* et al. Clinicopathologic and prognostic factors in adenoid cystic carcinoma of head and neck minor salivary glands: A clinical analysis of 130 cases. Am J Otolaryngol 2017; 38(2): 157–62.
6. *Liu HX, Wu M, Sun YM, Han FY, Liu YF, Zhang GZ*. Prognostic value of human papillomavirus infection and p53, p16, epidermal growth factor receptor and p34cdc2 expression in patients with salivary adenoid cystic carcinoma. Int J Clin Exp Pathol 2017; 10(7): 7882–9.
7. *Acharyaa S, Annehosurb V, Hallikeri K, Shivappaa KS*. Adenoid cystic carcinoma of the sublingual salivary gland: Case report of a rare clinical entity. J Oral Maxillofac Surg Med Pathol 2016; (28): 88–94.
8. *Thierauf J, Ramamurthy N, Jo YV, Robinson H, Frazier PR, Gonzalez J,* et al. Clinically Integrated Molecular Diagnostics in Adenoid Cystic Carcinoma. Oncologist 2019; 24(10): 1356–67.
9. *Wong SHM, Kong WY, Fang CM, Loh HS, Chuah LH, Abdullah S,* et al. The TRAIL to cancer therapy: Hindrances and potential solutions. Crit Rev Oncol Hematol 2019; 143: 81–94.
10. *Gilmore A, King L*. Emerging approaches to target mitochondrial apoptosis in cancer cells. F1000Res 2019; 8: F1000 Faculty Rev–1793.
11. *Noori AR, Hosseini ES, Nikkhah M, Hosseinkhani S*. Apoptosome formation upon overexpression of native and truncated Apaf-1 in cell-free and cell-based systems. Arch Biochem Biophys 2018; 642: 46–51.
12. *Dorstyn L, Akey WC, Kumar S*. New insights into apoptosome structure and function. Cell Death Differ 2018; 25(7): 1194–208.
13. *Bratton SB, Salvesen GS*. Regulation of the Apaf-1-caspase-9 apoptosome. J Cell Sci 2010; 123(Pt 19): 3209–14.
14. *Acehan D, Jiang X, Morgan DG, Heuser EJ, Wang X, Akey* WC. Three-dimensional structure of the apoptosome: implications for assembly procaspase-9 binding and activation. Mol Cell 2002; 9(2): 423–32.
15. *Dai DL, Martinka M, Bush JA, Li G*. Reduced Apaf-1 expression in human cutaneous melanomas. Br J Cancer 2004; 91(6): 1089–95.
16. *Leo C, Richter C, Horn LC, Schütz A, Pilch H, Höckel M*. Expression of Apaf-1 in cervical cancer correlates with lymph node metastasis but not with intratumoral hypoxia. Gynecol Oncol 2005; 97(2): 602–6.
17. *Zlobec I, Minoo P, Baker K, Haegert D, Khetani K, Tornillo L,* et al. Loss of APAF-1 expression is associated with tumour progression and adverse prognosis in colorectal cancer. Eur J Cancer 2007; 43(6): 1101–7.
18. *Paik SS, Jang KS, Song YS, Jang SH, Min KW, Han HX*, et al.. Reduced expression of Apaf-1 in colorectal adenocarcinoma correlates with tumor progression and aggressive phenotype. Ann Surg Oncol 2007; 14(12): 3453–9.
19. *Sträter J, Herter I, Merkel G, Hinz U, Weitz J, Möller P*. Expression and prognostic significance of APAF-1, caspase-8 and caspase-9 in stage II/III colon carcinoma: caspase-8 and caspase-9 is associated with poor prognosis. Int J Cancer 2010; 127(4): 873–80.
20. *Yamamura M, Sato Y, Takahashi K, Sasaki M, Harada K*. The cyclin-dependent kinase pathway involving CDK1 is a potential therapeutic target for cholangiocarcinoma. Oncol Rep 2020; 43(1): 306–17.
21. *Geng X, Sun YY, Fu JJ, Cao L, Li Y*. Role of miR-155-5p expression and its involvement in apoptosis-related factors in thyroid follicular carcinoma. J Clin Pharm Ther 2020; 45(4): 660–5.

Received on October 26, 2020
Accepted on November 24, 2020
Online First December 2020