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**Review Article**

# Comparison of miRNA Profiles of Primary Tumors and Metastatic Tumors of Salivary Gland Tumors and their Role in Prognosis: A Systematic Review

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**Abstract**

**Background:** MicroRNAs (miRNAs) are implicated in several biological processes, such as control of tissue homeostasis, cell signaling, differentiation, proliferation, neoplastic transformation, and activation/inhibition of apoptotic mechanisms. In this systematic review, we evaluated the changes in the expression pattern of miRNAs in salivary gland tumors (SGTs).

**Methods:** A comprehensive search was conducted in PubMed, and Scopus with no language and date restrictions in Feb 2023. All the studies on SGTs that evaluated miRNA profiling were included. Relevant data regarding the overexpression and down-regulation of the miRNAs were extracted. The quality of the included studies was evaluated with Newcastle–Ottawa checklist. The altered expression of miRNAs was evaluated between SGTs and normal cases, benign and malignant tumors, and primary and high-grade tumors.

**Results:** Thirteen studies were included in this systematic review. There were considerable differences between malignant and benign tumors regarding the miRNAs expression level. In the five studies, the miRNA profile of the primary tumors was compared with metastatic tumors to reveal the involvement of the miRNA in the prognosis of the salivary tumors. The miRNAs expression changes were correlated with tumor size, stage, recurrence, and occurrence of solid components. Perineural invasion and lymph node metastasis were also reported in ACC-LM cell line and recurrence of adenoid cystic carcinoma (ACC) tissues.

**Conclusion:** The miRNA profiling confirms their prognostic value in salivary gland tumors. Significant alternations of the miRNAs expression are useful for distinguishing different types of salivary tumors and malignant tumors from benign types. The miRNA expression changes also affect the prognosis of salivary tumors.

**Keywords:** Salivary gland neoplasms; MicroRNAs; Neoplasm metastasis; Biomarkers; Systematic review

## Introduction

Salivary gland tumors (SGTs) are large and diverse groups of benign and malignant neoplasms. The incidence of salivary gland tumors is approximately 3%-6% of all head and neck tumors, and they are a relatively uncommon heterogeneous group of neoplasms (1). There are significant clinical, morphological, and histological differences among SGTs that can lead to differential diagnosis and misleading information. The rarity of salivary tumors, diverse tumor subtypes, and the overlapping clinicopathological characteristics of the tumor subtypes lead to diagnostic challenges for the pathologist. There are almost 24 different subtypes of SGTs; pleomorphic adenoma (PA) is the most common benign salivary tumor whereas, mucoepidermoid carcinoma (MEC) and adenoid cystic carcinoma (ACC) are classified as malignant salivary gland tumors (2). MEC is mainly observed in pediatric patients, and ACC is associated with poor prognosis, perineural invasion, and distant metastasis of the lung, liver, or other organs. Surgical resection is the routine management of salivary tumors, and there are several preoperative diagnostic approaches including clinical examinations and imaging tools with or without fine needle aspiration cytology (FNAC) (3). The importance of salivary biomarkers as novel non-invasive tools for diagnosing cancer and systemic disease has been noticed recently in conditions ranging from oral diseases to systemic diseases (4-6).

MiRNAs are a class of small, non-coding RNAs (17-25 nucleotides) that bind to the 3′UTR of target mRNA to regulate mRNA and adjust the protein level (7, 8). The miRNAs are implicated in several biological processes, such as control of tissue homeostasis, cell signaling, differentiation, proliferation, neoplastic transformation, and activation/inhibition of apoptotic mechanisms. Changes in the expression pattern of miRNAs can affect the tissue environment, tumoral cell progression and vascularization. Therefore, miRNAs might function as either oncogene or tumor suppressor under certain circumstances, and altered expression of miRNAs is involved in physiological and pathological conditions such as infectious diseases and cancer development (9-11). MiRNAs have been used as tissue-based prognostic markers and for the classification of malignancies, including breast, prostate, colon, pancreas, and thyroid cancers (12-16). Positive association has been previously observed between miRNA overexpression with the presence of lymph node metastasis, tumor size, and lung metastasis in SGTs. Lymph node metastasis and clinical prognosis affect the survival of patient (17).

Therefore, we aimed to systematically review the studies on miRNAs profiling of different types of SGTs to consider altered expression (up-regulation and down-regulation) of miRNAs and their prognostic effect in malignant tumors compared with benign types.

## Materials and Methods

The Preferred Reporting Item for Systematic Reviews and Meta-analysis (PRISMA) statement was used to guide the present work (18).

### *Search strategy*

A comprehensive search was performed through two databases of PubMed and Scopus. The following search strategy was used in each database: RNA AND (salivary gland tumor OR salivary gland carcinoma OR salivary adenocarcinoma). Screening of the databases was done in Feb 2023. A comprehensive search was performed by two authors, and for those articles on which the authors disagreed, the opinion of another author was sought. If the disagreements could not be resolved, the consensus of all three authors was used.

We did not have any language or publication date restriction in our search strategy. The title and abstract of initial search results were screened by two

independent investigators using End-Note, for all relevant studies published. Duplicate articles were omitted. The full texts of the remaining articles were evaluated to exclude the irrelevant articles. The reference lists of identified studies were reviewed for relevant articles not picked up from the search strategy.

### *Inclusion and exclusion criteria*

Inclusion criteria were all the studies specifically reporting on miRNA profiling and expression level of different miRNAs of malignant and benign salivary gland tumors. Case reports, conference proceedings, personal communications, letters to editor, experimental articles, reviews, and articles not reporting the miRNA profile of the SGTs were all excluded.

### *Data Extraction and Quality Assessment*

Data were extracted independently by two authors. Information extracted from each study were summarized into an Excel table (Office Suite, Microsoft Corp.), which considered: The details of the study (title, authors, publication year, and country), case, control, miRNA profiling method, type of salivary gland tumor examined, up-regulated and down-regulated miRNAs. Two reviewers independently assessed the quality with Newcastle–Ottawa and extracted data in the Pre-defined checklist. Quality of our selected articles was evaluated with eight-item Newcastle–Ottawa (NOS) checklist for case–control studies. This instrument has three domains: 1) selection of participants, 2) comparability, and 3) outcome ascertainment.

## Results

The process of searching and selecting relevant studies has been described in Fig. 1. In total, 19 studies were included in this systematic review as the most relevant articles. In 13 studies, miRNA profile of the benign and malignant salivary tumors was compared with the normal tissue sample (19-28).

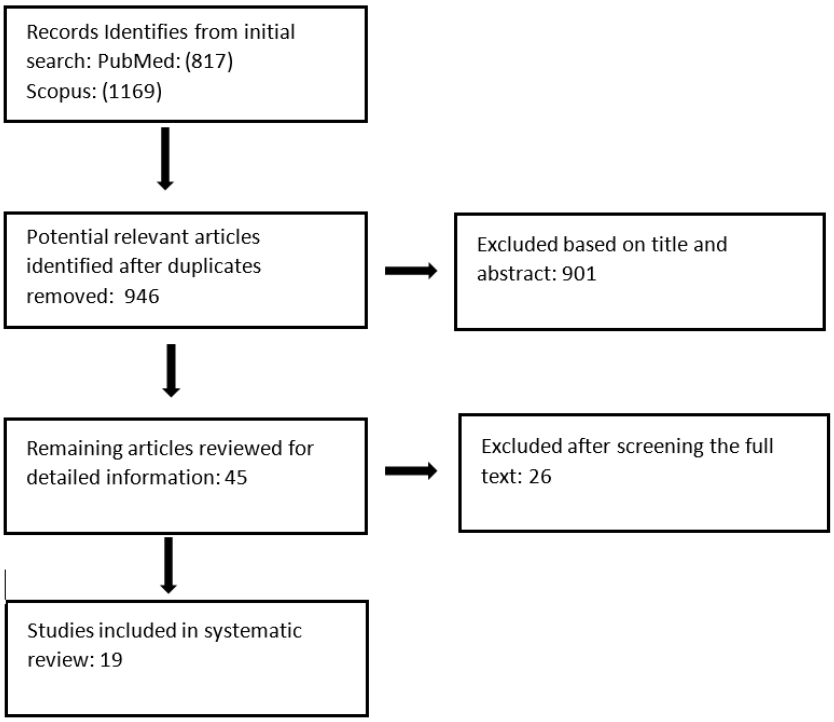


**Fig. 1:** Flowchart for the search strategy and selection process

The miRNA profile of the PA tissue samples was compared with normal tissue in 3 of the studies (25, 29). MiRNA profile of the MEC tissue was compared with the normal tissue in three of the studies (20, 25, 28). The majority of the studies (6/10) evaluated miRNA profile of the ACC tissue samples with the normal tissue (19, 21, 23-25, 27). Only one study evaluated the miRNA profile of the warthin tumor in comparison with normal salivary tissue (22). Ten studies used tissue samples, two studies examined cell lines, and one study used plasma sample for comparing the miRNA profile of the benign and malignant salivary tumor with normal cases. The study of Cinpolat et al. evaluated the miRNA expression profile on serum, saliva, and tissue samples of the patients (22). Summarized characteristics of the studies evaluated miRNA profile of malignant or benign samples in comparison with normal samples are presented in Table 1. The miRNA profiling was performed by miRNA microarray followed by RT-PCR for validating the results in most of the studies.

**Table 1:** Characteristics of the articles compare miRNA profile in salivary gland tumors compared with normal cases

| *Author/ year* | *Number Case/Control* | *Sample* | *Method* | *MiRNA profile change* | *Most highly changed* | |
|---|---|---|---|---|---|---|
| | | | | | Up regulated miRNA | Down regulated miRNA |
| Zhang/ 2009 Australia (29) | PA :17 Normal:17 | Tissue | Microarray data analysis and qRT-PCR | Up-regulated: 17 Down-regulated: 5 | miR-140, miR-229-3p, miR-29c miR-302c, miR-376a | let-7a, miR-375 |
| Cinpolat/2017 Turkey (22) | Warthin tumor: 9 PA: 7 Normal: 17 | Plasma | qRT-PCR | Up-regulated: 0 Down-regulated: 8 | No | miR-21, miR-23a, miR-27a miR-223, miR-125b, miR-126, miR-146a, miR-30e |
| Binmadi/2018 USA (20) | MEC:6 Normal:3 | Tissue | The TaqMan Human miRNA Cards Array and qRT-PCR | Upregulated: 63 Downregulated:5 | miR-302a, miR-490 miR-21 miR-22 miR-205 | miR885-5p, miR-375 miR-19a, miR-363-3p miR-192 |
| Naakka/2022 Brazil (28) | MEC: 25 Normal: 6 | Tissue | MiRNA expression analyses and qRT-PCR | Upregulated: 18 Downregulated:28 | miR-21-5p, miR-22-3p, miR-181a-5p miR-205-3p miR-224-3p | miR-363-3p, miR-625-5p miR-885-5p miR-892b miR-1288-3p |
| Mitani/ 2013 USA (24) | ACC:13 Normal:4 | Tissue | Microarray and qRT-PCR | Upregulated: 19 Downregulated:36 | miR17-92 cluster miR-455-3p, -455-5p, miR-181 miR-183 miR106 b-25 miR106a-363 | miR-375, miR-142-3p miR-142-5p, miR-148, miR-155, miR-33b miR-29c, miR-14 miR-205 |
| Andreasen/2018 Denmark (19) | primary ACC:11 Normal: 11 | Tissue | MicroRNA array | Upregulated: 1 Downregulated:7 | miR-1271-5p | miR-1199-3p, mir-4717-5p miR-610, miR-68785p miT-519, miR-5572 |

**Table 1:** Continued….

| | | | | | | |
|---|---|---|---|---|---|---|
| Andreasen/2018 Denmark (19) | Metastatic ACC:11 Normal:11 | Tissue | MicroRNA array | Upregulated: 4 Downregulated:7 | miR-922, mir-1271-3p miR6790, miR-6894 | miR-6865, miR-1199-3p miR4717-5p, miR499a miR-4790, miR3936 miR-127 |
| Andreasen/2018 Denmark (30) | Primary and metastatic ACC:64 Normal:10 | Tissue | The Affymetrix miRNA 4.1 array platform | Upregulated:22 Downregulated:25 | MiR-181d-5p MiR-455-5p | miR-148a-5p miR-885 miR-29c-3p miR-139 |
| Han/2018 China (23) | ACC: 6 Normal:6 | Tissue | HT-NGS technology, qRT-PCR | Upregulated:15 Downregulated:25 | miR-520a-5p, miR-445-5p miR-181b | miR-885-5p, miR-375 miR-20b-5p |
| Kiss/2015 Hungary (27) | ACC:2 Normal:1 | Tissue | Affymetrix miRNA Array | Upregulated:7 Downregulated:9 | miR-17 miR-20a | Let-7b miR-193b |
| Matse /2015 China (31) | SGTs: 12 Contralateral Normal tissue:12 | Parotid Saliva | RT-qPCR | Upregulated:1 Downregulated:6 | Has-miR103a-3p | Has-miR-211 Has-miR-296-5p Has-miR-425-5p Has-miR-1233 Has-miR1267 Has-miR1825 |
| Boštjančič/2017 Slovenia (17) | SGTs:70 Normal:11 | Tissue | Rt-qPCR | Upregulated:2 Downregulated:1 | MiR-133b miR- 99b | miR-140 |
| Gao/2014 USA (32) | ACC:12 Normal:12 | Tissue | Microarray assay qRT-PCR | Upregulated:11 Downregulated: 11 | miR-455-3p miR-181b/a miR-146a miR-106b | miR-375 miR-31 miR-150 miR-152 |
| Santos/2017 Brazil (25) | ACC: 2 Normal: 11 | Tissue | TaqMan MicroRNA RT Reverse Transcription Kit and qRT-PCR | Upregulated:1 Downregulated: 5 | miR-9 | miR-16 miR-221 miR-122 miR-195 |
| Santos/2017 Brazil (25) | PA: 2 Normal: 11 | Tissue | TaqMan MicroRNA RT Reverse Transcription Kit and qRT-PCR | Upregulated:1 Downregulated: 4 | miR-9 | miR-221 miR16 miR132 miR-17 |
| Santos/2017 Brazil (25) | MEC: 2 Normal:9 | Tissue | TaqMan MicroRNA RT Reverse Transcription Kit and qRT-PCR | Upregulated:0 Downregulated: 3 | NO | miR-9 miR-16 miR-221 |

Adenoid cystic carcinomas (ACC), mucoepidermoid carcinomas (MEC) and pleomorphic adenomas (PA), SAM: Significant analysis of microarray, ACC-M: a highly metastatic SACC cell line

In four studies, miRNA profile of the malignant salivary gland tumors was compared with benign salivary tumors using tissue, plasma, and saliva. Santos et al. obtained no significant difference between malignant and benign tumors regarding the overexpression of the miRNAs (25). Cinpolat et al. showed no significant difference between malignant and benign tumors regarding down-regulation of the miRNAs (22). Characteristics of the studies on malignant versus benign salivary tumor miRNA profile differences are summarized in Table 2.

**Table 2:** Characteristics of the articles evaluated the miRNAs profile of the malignant salivary tumor compared with benign salivary tumors

| *Author* | *Comparison* | | *Method* | *Malignant vs. benign* | |
|---|---|---|---|---|---|
| | Malignant | Benign | | Up-regulated | Down-regulated |
| De-naro/2019 Italy (33) | MEC:6<br>ACC:4<br>ACC*:1<br>SDC:1<br>cystadenocarcinoma:1<br>adenocarcinoma:1 | PA: 8 | nCounter human miRNA expression assay | N: 32<br>miR-17-92 cluster (miR-93, miR-106a, miR106-b, miR-20a)<br>miR-21-5p, miR-181a-5p<br>miR-199a-5p, miR-4286<br>let-7g-5p, miR-17-5p, miR-199a(b)-3p, miR-15b-5p<br>miR-126-3p, miR-126-3p<br>miR-320e, miR-19b-3p<br>miR-200b-3p, miR-222-3p<br>miR-146a-5p, miR-374a-5p<br>miR-1246, miR-150-5p | N:29<br>miR-376c-3p, miR-125b-5p<br>miR-376a-3p, miR-127-3p<br>miR-136-5p, miR-495-3p<br>let-7e-5p, miR-377-3p<br>miR-99b-5p, miR-100-5p<br>miR-382-5, miR-195-5p<br>miR-582-5p, miR-411-5p<br>miR-379-5p, miR-154-5p<br>miR-543, miR-135a-5p<br>miR-27b-3p, miR-9-5p, miR-140-5p |
| Cinpo-lat/2017 Turkey (22) | Primary SCC:2<br>High grade MEC:1<br>ACC:1 | Warthin tumors: 9<br>PA:7 | Tissue MicroRNA isolation<br>qRT-PCR | N:5<br>miR-21, MiR-146b<br>MiR-31, MiR-345<br>MiR-199a, | - |
| Cinpo-lat/2017 Turkey (22) | N:4<br>SCC:2<br>MEC:1<br>ACC:1 | N:14<br>Warthin tumors: 9<br>PA:7 | Plasma MicroRNA isolation<br>qRT-PCR | miR-199a<br>miR-30e | - |
| Matse/2013 USA (34) | SCC:9<br>Adenocarcinoma NOS:2<br>ACC*:3<br>MEC:3<br>SDC:3<br>Undifferentiated carcinoma:1<br>Carcinoma ex-pleomorphic adenoma:1<br>ACC:2<br>Myoepithelial carcinoma:1<br>BCC:1 | PA:13<br>Warthin tumor:5<br>Basal cell adenoma:1 | Saliva TaqMan microRNA assays<br>qRT-PCR | mmu-miR-140-5p, miR-374, miR-222, miR-15b, let-7g, miR-132, miR-140-5p<br>hsa-let-7g, miR-132, miR-519b-3p, miR-223, -miR-30a-3p | hsa-miR-519b-3p<br>hsa-miR-520C-3p<br>hsa-miR-520D-3p |
| Kim/2022/korea (35) | CXPA CA portion (13)<br>four SDC: 4, epithelial-myoepithelial carcinoma: 2 MEC: 2 Adenocarcinoma NOS: 3<br>Clear cell carcinoma: 1<br>Oncocytic carcinoma: 1 | CXPA PA portion (13) | miRNA sequencing | miR-21-3p, miR-183-5p<br>miR-182-5p, miR-425-5p<br>miR-96-5p, miR-200a-3p<br>mir-181a-3p, miR-505-3p | miR-455-3p, miR-140-5p<br>miR-483-5p, miR-125b-5p<br>miR-125a-5p |
| Kim/2022/Korea (35) | CXPA (13) | Benign PA (16) | miRNA sequencing | miR-196a-5p, miR193a-3p<br>miR193b-3p, miR-29c-3p<br>miR-331-3p, miE361-3p | Let-7a-3p, miR-27a-3p<br>miR-9-5p, miR-135a-5p<br>miR-455-5p, miR218-5p<br>miR181d-5p, miR369-3p<br>miR132-5p |

ACC*: Acinic cell carcinoma, SDC: Salivary duct carcinoma, SCC: Squamous cell carcinoma, BCC: Basal cell carcinoma, CXPA: Carcinoma ex pleomorphic adenoma, CA: carcinomatous portion, PA: pleomorphic adenoma.

To reveal the involvement of the miRNA in the prognosis of the salivary tumor, miRNA profile of the primary or benign salivary gland tumor was compared with miRNA profile of the metastatic and high-grade tumor, in five of the studies (19, 24, 28, 36). According to these studies, altered expression of the miRNAs is correlated with tumor size, stage, recurrence, and occurrence of solid components. Perineural invasion and lymph node metastasis were also reported in ACC-LM cell and recurrence ACC tissues by two authors (24, 36). Andreasen et al reported no correlation between miRNA expression and stage of the ACC (19). In Table 3, data of the studies on miRNA profiling of the metastatic salivary gland tumor with poor survival compared with primary tumor are summarized.

Data regarding the quality assessment of the included studies based on the Newcastle Ottawa scale are presented in Table 4.

**Table 3:** Studies evaluated altered miRNA expression changes of the salivary gland tumor with worse survival

| *Author* | *Samples* | *Up-regulated* | *Down-regulated* | *Clinicopathologic changes* |
|---|---|---|---|---|
| Naakka/2022<br>Brazil (28) | High grad MEC (N:9) vs. low (N:20) or intermediate (N:7) grade MEC | miR-205-5p<br>miR-224-5p | miR-582-5p<br>miR-3125<br>miR-4324<br>miR-139-3p<br>miR-145-3p<br>miR-148a-3p<br>miR-186-5p<br>miR-338-3p<br>miR-363-3p | Invasion and migration |
| Feng/2017<br>USA (36) | SACCLM vs. SACC83 | miR-99a-5p<br>miR-155-5p | miR-130a-3p<br>miR-342-3p<br>miR-205-5p | Perineural invasion |
| Feng/2017<br>USA (36) | Recurrent ACC (6) vs. primary ACC (6) | miR-155-5p<br>miR-342-3p | miR-205-5p | Perineural invasion |
| Andreasen/2018<br>Denmark<br>Genetic (30) | Metastatic ACC (22) vs. primary ACC (25) | No difference | No difference | Not reported |
| Chen/<br>2014<br>China (21) | ACC-2 vs.<br>ACC-M | N: 20<br>miR-4487<br>miR-4430<br>miR-5096<br>miR-1285-3p | N: 18<br>miR-5191<br>miR-3131<br>miR-4278<br>miR-4498 | Not reported |
| Wang/2018<br>China (37) | ACC-M vs.<br>ACC-2 | miR-146a-5p/3p | miR-361-5p/3p<br>miR-338-5p/3p | - |
| Andreasen/2018<br>Micro<br>Denmark (19) | Tissue<br>ACC<br>Validation group:120 | hsa-mir-21,<br>hsa-mir-181a-2<br>hsa-mir-152<br>mir-4676<br>has-mir-6835-3p | miR-374c<br>miR-1180 | |
| Mitani/2013<br>USA (24) | ACC tissue<br>Patients died:8 vs.<br>Alive patients:22 | miR-17-92 cluster (miR-17, and -20a)<br>let-7a<br>miR-150<br>miR-9 | - | Tumor size (108 miRNA)<br>Tumor stage(18 miRNAs)<br>Tumor recurrence (39 miRNAs)<br>Lymph node metastasis (13 miRNAs)<br>Solid component |

SACC-83: adenoid cystic carcinoma cell lines with low lung metastasis rate. SACC-LM daughter cell line of SACC-83 with high lung metastasis.

**Table 4:** Quality control of the included studies.

| *Author/ year* | *Adequate definition of case* | *Representative of cases* | *Selection of control* | *Definition of control* | *Comparability of cases and controls on the basis of the design or analysis* | *Same method of ascertainment for cases and controls* |
|---|---|---|---|---|---|---|
| Zhang/ 2009(29) | * | * | * | * | * | * |
| Cinpolat/2017(22) | * | * | * | * | * | * |
| Binmadi/2018(20) | * | * | * | * | * | * |
| Naaakka/2022(28) | * | * | * | * | * | * |
| Chen/2014(21) | * | * | * | * | * | * |
| Mitani/2013(24) | * | * | * | * | * | * |
| Andreasen/2018(30) | * | * | * | * | * | * |
| Andreasen/2018(19) | * | * | * | * | * | * |
| Matse/2015 (31) | * | * | * | * | * | * |
| Matse/2013 (34) | * | * | * | * | * | * |
| Boštjančič /2017 (17) | * | * | * | * | * | * |
| Han/2018(23) | * | * | * | * | * | * |
| Kiss/2015(27) | * | * | * | * | * | * |
| Denaro/2019(33) | * | * | * | * | * | * |
| GAO/2014(32) | * | * | * | * | * | * |
| Kim/2022(35) | * | * | * | * | * | * |
| Wang/2018 (37) | * | * | * | * | * | * |
| Feng/2017(36) | * | * | * | * | * | * |
| Santos/2017(25) | * | * | * | * | * | * |

## Discussion

The current study showed that miRNA expression patterns have a role in the tumorigenesis of benign and malignant salivary gland tumors.

### *Up-regulated miRNAs*

The up-regulation of the miR-376a, miR-301, and miR-21 have been reported in cervical and pancreatic cancers; similar increased expression was detected in benign salivary tumors of PA compared with healthy tissue (26, 38, 39). The miR-21 is one of the most prominent miRNAs that is up-regulated during cancer progression and is associated with poor prognosis. The over-expression of the miR-21 has been mentioned in six of the studies that evaluated PA, MEC, and ACC tissues miRNAs compared with normal salivary tissue (20, 22, 26, 28, 33, 40); whereas, in the study of Cinpolat et al, decreased expression of the miR-21 was observed in plasma sample of the PA cases compared with normal ones (22). Despite the decreased expression of the mir-21 in plasma samples of the PA than normal cases, significantly increased expression of the miR-21 was observed in malignant PA tissue samples compared with benign PA tissue samples. Aberrant expression of miR-21 has been reported in previous studies as a possible oncogene and a key determinant of malignant progression and metastases (41-43). MiR-21 has also been reported as a stable miRNA that is overexpressed in head and neck squamous cell carcinomas (SCCs) with the following characteristics of

anti-apoptotic effects, promoting cell invasion, proliferation, and metastases (44). Therefore, suppression of miR-21 may provide a potential approach for treating advanced salivary gland tumors. Similar expression of miR-30e to miR-21 might be due to their role in tumor development. MiR-30e is another biomarker that was over-expressed in plasma samples of malignant salivary tumors compared with benign tumors, and down-expressed in plasma samples of benign salivary tumors (PA) compared to controls (22).

MiR-302a, miR-544, miRNA-205, and miRNA-22 were the most overexpressed miRNAs in MEC salivary gland tumors evaluated in the included studies (20, 28). Based on the studies, overexpression of the miRNAs in MEC is involved in irregular gene expression and cell invasion during cancer tumorigenesis and progression. MECs have various clinicopathological behavior, so detecting the disease prognosis and decision for further treatment is challenging (45).

Although miR-205 was increased in MEC primary and metastatic tissues compared with normal control tissue (20, 28), studies on ACCs cell lines with lung metastasis and recurrent ACC tissue showed significant reduction in miR-205 expression compared with primary ACC and ACC cell line without metastasis (36).

In the study of Cinpolat et al., comparing tissue and plasma samples of malignant salivary tumors of ACC, MEC, and SCC with benign salivary tumors of warthin tumors and PA, showed increased expression of the miR-199a, miR-146b, and miR-31 (22). Their results were confirmed by the study of Denaro et al. on malignant tumors miRNA profiling (33). The prognostic value of these miRNAs has been shown in melanoma, prostate, thyroid, colon, hepatocellular, and gastric cancers. Based on these studies, increased expression of the miR-146b, 31, and 199a is associated with decreased survival rate, so confirm their prognostic value (13, 46-50).

The overexpression of miRNAs such as miR-455 in salivary gland tissues is proposed a complementary tool to diagnose challenging cases of ACC and MEC (24, 51). Further investigations are needed to reveal the functional role of miR-455 in tumorigenesis and progression of the ACCs. Since there is a diagnostic challenge for ACCs, mir-455 is potentially involved in ACCs pathogenesis and have diagnostic potential.

Different expression of the miRNAs between malignant and benign tumors has been evaluated in four studies (22, 25, 33, 34). The expression of the miR-9 was examined in the study of Santos et al. who observed no significant difference of miR-9 expression between malignant ACC and MEC salivary tumors compared with PA. Similar expression levels of the miR-9 between malignant and benign tumors might be related to the controversial effect of miR-9 across tumor types (25). Increased expression of the miR-15b in malignant salivary tumors compared with benign types was also associated with tumorigenesis and poor prognosis reported by two groups. Overexpression of miR-15b could block the apoptosis pathway and reduce the expression of reversion-inducing cysteine-rich protein with Kazal motifs (RECK) gene in colorectal and gastric cancers (33, 34).

### *Down-regulated miRNAs*

We identified that significantly decreased expression of some miRNAs was related to high-grade salivary tumors and could be implicated in tumorigenesis and invasion of cancers. Zhang et al. reported dramatically decreased expression of miR-144, miR-140, miR-375, and let-7 in benign salivary gland tumors of PA (29). Reduced expression of the mentioned miRNAs such as let-7 has been previously reported in lung cancer (52, 53). The miRNA-885-5p, miRNA-375, miR-363-3p, and miR-4324 were among the miRNAs that revealed significant down-expression in malignant salivary gland tumor of MEC and ACC when compared with the normal tissue (20, 23, 24). These miRNAs are putative prognostic biomarkers that are down regulated following tumor progression (20, 28). The decreased expression of the miR-375 has been confirmed in other cancerous tissues, such as gastric carcinoma, and renal and pancreatic cancers. Based on three of the included studies, down-regulation of miR-885-5p plays an important role in carcinogenesis of the salivary gland adenoid cystic

carcinoma (SACCs), and the SCUBE3 mRNA was reported as its primary target (20, 23, 28). Let-7a and b are a member of the miRNA family involved in both normal development and cancer progression and has oncogenic effect following their overexpression. However, loss of expression of let-7a is shown to be associated with the development and progression of the cancers (27, 29).

MiR-125b was also among the miRNAs that showed down-regulation not only in plasma samples of the PA cases compared with plasma of the normal cases (22), but also in tissue samples of malignant salivary gland tumors compared with benign types tissue samples (33). Normal expression of miR-125 has been reported as a tumor suppressor in hepatocellular, breast and gastric cancers; however, the dysregulation of the miR-125 has resulted in alternations in chromosome 11 that lead to salivary gland MEC, head and neck SCC, oral cancers, and metastasis in many tumors (54-56).

The study of Liang et al. on salivary ACC cell lines, showed that lower level of miR-125 is positively associated with metastatic phenotype (57). Down-regulation of some miRNAs was also observed in SACC tissues; however, the correlation between down-regulation of these miRNAs and aggressiveness of the tumor behavior is not apparent (24). Down-regulation of miR-148 in ACC compared to normal tissue reported by two of the studies was similar to its decreased expression in high-grade MEC versus normal or intermediate-grade and primary MEC compared to normal tissue, reported by Naakka et al. MiRNA-148 expression acts as a tumor suppressor by inhibiting cell proliferation, growth, migration, and invasion (28). However, down-regulation of the miRNA-148 increases the proliferation, invasion, and metastasis of tumor cells through different target genes (58). Several underlying mechanisms might be responsible for oncogenic effect of the down-regulation of miRNAs, including overexpression of the PLAG1, RAS oncogene, and HMGA2 (42, 59). Based on the studies on PA as the most frequent benign salivary gland tumor, evaluating the miRNA profile has diagnostic value with 100% accuracy for discriminating pleomorphic adenoma from the normal tissue (29). Recent studies have documented the significant association between the miRNA gene's location to fragile sites, and genomic regions involved in cancers that can affect the proliferative signaling, cell death, activating invasion and metastasis (60, 61). Deregulation of the miRNA expression might be the consequence of various mechanisms, such as amplification or deletion of miRNA genes, abnormal transcriptional control of miRNA, deregulated epigenetic changes, chromosomal abnormalities, and defects in miRNA biogenesis machinery.

### *Prognostic value of miRNA*

Dysregulation of the miRNA expression has been observed in metastatic or high-grade salivary gland tumors which shows the involvement of the miRNA in progression of cancer and poor prognosis of the disease. The study of Chen et al confirmed the association between altered expression of miRNAs (upregulation of miR-4487, miR-4430, and miR-486-3p) and hematogenous spread of SACC to lungs using miRNA microarray and qPCR analysis, which might be helpful in providing a novel molecular therapeutic target for the treatment of SACC (21). MiR-17-92 cluster has shown an important role in progression of different tumor types. The miR-17-92 cluster (miR-17 and miR-20, miR-93, miR-106) overexpression revealed statistically significant association with aggressiveness of tumor behavior and poor survival of ACC, MEC, and SCC compared with benign salivary tumors (24, 33). Mitani et al obtained significant relation between upregulation of the miR-20a, -17, and -9 and occurrence of solid components in ACC tissues. They also reported positive association between overexpression of let-7a and miR-150 with size, stage, lymph node metastasis, and recurrence of the ACC (24). The overexpression of miR-133 has also shown positive relation with tumor size and poor prognosis in salivary tumors compared with healthy tissue (17).

MiR-155 was evaluated in three studies, and showed upregulation in salivary ACC with lung metastasis cell lines and tissue samples compared with primary ACC (36); however, in comparison with healthy controls, miR-155 expression was decreased in ACC tissue samples (24). The increased

expression of the miR-155 was associated with perineural invasion through increased expression of the UBA2 gene as a mediator of the SACC metastasis.

MiR-582-5p and -4323 were another miRNA that showed down regulation in high grade MEC compared with low grade MEC and were influential on invasion and migration of the tumor cells through the target genes of EZH2, PIK3CA, and PTEN. High-grade MEC showed decreased expression of miR-582-5p and miR-4324 compared with intermediate/low grade MEC associated with shorter overall survival and worse prognosis by overexpression of the EZH2 and PTEN target genes. The overexpression of EZH2 was previously reported in MEC, myoepithelial carcinoma of salivary glands, and ACC. In another study performed by Andreasen et al., miRNAs were differentially expressed between primary ACC and metastases as compared to normal salivary gland tissue. However, there was no difference between primary and metastatic lesions, and they reported no correlation between miRNA profile expression and tumor stage, and obtained no prognostic value for altered miRNAs expression (19). The inconsistent results showed that primary ACC have inherent metastatic capacity and are similar to their metastatic forms regarding the miRNA profile (30).

The major limitation of the current study was small sample size of most of the studies and low number of studies with follow up duration to evaluate the miRNA profile in recurrence tumors and metastatic tissues. Larger cohorts with long term follow up are needed increase the accuracy of the results.

## Conclusion

miRNA profiling confirms their prognostic value in salivary gland tumors. Significant alternations of the miRNAs expression are useful for distinguishing different types of the salivary tumors and malignant tumors from benign types. MiRNA expression changes also affect the prognosis of the salivary tumors. However, further studies are needed to obtain consistent results and reveal the specific diagnostic and prognostic values of miRNAs.

## Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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## Conflict of interest

The authors declare that there is no conflict of interests.

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