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Epigenetic alterations in salivary gland neoplasms and the impact of these alterations in tumor progression and prognosis: A systematic review and meta-analysis

Karolina Skarlet Silva Viana [a,1], Mariana Saturnino de Noronha [b,1], Cristiane Squarize [c], Lucas Guimarães Abreu [d], Maria Cássia Ferreira Aguiar [a], Vanessa Fátima Bernardes [e,*]

[a] Department of Oral Surgery and Pathology, School of Dentistry, Universidade Federal de Minas Gerais, Antônio Carlos Avenue, 6627, Pampulha, Belo Horizonte, MG, Brazil, 31.270-901
[b] Faculdade Sete Lagoas, Itália Pontelo Street, 86, Centro, Sete Lagoas, MG, Brazil, 35700-170
[c] Department of Periodontics and Oral Medicine, University of Michigan School of Dentistry, Ann Arbor, MI, USA
[d] Department of Child's and Adolescent's Oral Health, School of Dentistry, Universidade Federal de Minas Gerais, Antônio Carlos Avenue, 6627, Pampulha, Belo Horizonte, MG, Brazil, 31.270-901
[e] Department of Pathology, Biological Sciences Institute, Universidade Federal de Minas Gerais, Av. Presidente Antônio Carlos, 6627, 3rd Floor, Block G, Room 60, Belo Horizonte, MG, Brazil, 31.270-901

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ABSTRACT

Salivary gland tumors (SGTs) are uncommon lesions with etiologic factors not clearly defined. Epigenetic alterations have been suggested as one of the etiologic factors and as a possible factor impacting the prognosis of SGTs. The epigenetic alterations might include DNA methylation, alterations on the expression of noncoding RNAs and structural modification of histones. The aim of this study was to evaluate the available evidence about the epigenetic alterations associated to SGTs and their possible role on prognosis of SGTs. A Systematic Review was conducted according to PRISMA statement and prospectively registered on PROSPERO (CRD42022325647). The PECO question was "What are the epigenetic alterations in salivary gland neoplasms and their role in the progression and the prognosis of the tumor?". A search strategy was elaborated to retrieve studies able to answer this question and applied to each database: PubMed, Web of Science and Scopus, as well as the Grey Literature. Two independent reviewers worked in all steps. The search comprised a total of 1807 studies and after removal according to inclusion and exclusion criteria, 47 studies were included in this systematic review. The Joana Briggs Institute tool was used for appraisal of methodological quality of studies. A meta-analysis was conducted for methylation regarding the genes MGMT and RASSF1A and found that both genes methylation is related to higher stages of the SGTs but did not show an association with grade of tumors. The results must be evaluated carefully, once GRADE showed a very low certainty of evidence.

1. Introduction

Salivary gland tumors (SGTs) are uncommon lesions that show great variability in their microscopic and clinical features. According to World Health Organization, SGT represent 6 % of head and neck tumors [1]. The most common type of salivary gland tumor is pleomorphic adenoma, and regarding malignant salivary gland tumors, mucoepidermoid carcinoma represents the most frequent salivary gland malignancy [2]. SGTs present a diverse range of histological and clinical behaviors. The rarity of these tumors combined with the diverse histology means that there is a lack of studies that can be used to provide strong recommendations for each individual histologic subtype of salivary tumor [3].

The aetiologic factors for SGTs are not clearly defined. Nutrition may be a risk factor: low intake of vitamins A and C correlates with a high incidence of these tumors. Irradiation may also be cited as a possible cause [2,4] and epigenetic alterations have been suggested as one of the possible etiological factors [5].

* Corresponding author.
E-mail addresses: karolinasilvaviana@gmail.com (K.S.S. Viana), mariana.satnoronha@gmail.com (M.S. Noronha), csquariz@umich.edu (C. Squarize), lucasgabreu01@gmail.com (L.G. Abreu), cassiafaster@gmail.com (M.C.F. Aguiar), bernardesvf@icb.ufmg.br (V.F. Bernardes).
[1] Both authors contributed equally to this study.

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Abbreviations

SGTs	Salivary Gland Tumors
MEC	mucoepidermoid carcinoma
PA	pleomorphic adenoma
ACC	adenoid cystic carcinoma
IHC	immunohistochemistry

Epigenetic is defined as heritable changes in a cellular phenotype that are independent of alterations in DNA sequence. The information conveyed by epigenetic modifications plays a critical role in the regulation of all DNA-based processes, such as transcription, DNA repair and replication [6]. Consequently, alterations in this process can lead to the induction and maintenance of various tumors [7,8]. There are three mechanisms of epigenetic events: DNA methylation, alterations on the expression of noncoding RNAs, including microRNAs (miRNAs) and structural modification of histones [9].

It has been indicated that epigenetic alterations can be responsible for the development and progress of salivary gland neoplasms. These alterations have been studied in salivary gland neoplasms and were associated with development and progression of these, influencing prognosis [9,10].

Thus, this study aims to evaluate the existing evidence about the epigenetic alterations associated to SGTs and their possible role in the progression and prognosis of the tumor, as well as the possible use of these alterations as biomarkers to guide the treatment.

2. Methods

2.1. Study design

The Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) checklist was used as reporting guide for conducting this Systematic Review [11]. A prospective protocol for this study was registered on the International Prospective Register of Systematic Reviews in Health and Social Care (PROSPERO, National Institute for Health Research, UK) website under the number CRD42022325647.

2.2. Research question and outcome

The acronym PECOS was used to structure the research question as follows: "What are the epigenetic alterations in salivary gland neoplasms and their role in the progression and the prognosis of the tumor?"

P (Patients) = individuals with salivary gland neoplasia
E (Exposure) = epigenetic alterations
C (Comparator) = no epigenetic alterations
O (Outcomes) = prognosis of the tumor
S (Study design) = Studies with humans

The primary outcomes of this study were features associated with the prognosis of the tumor, such as: death, lymphovascular invasion, local recurrence, nodal metastasis, distant metastasis, tumor size, extra-parenchymal spread, soft tissue invasion, perineural invasion, glandular location, sub-location of tumors and grade.

2.3. Search strategy and information sources

Electronic searches were conducted in April 2022 across the following electronic databases: PubMed (National Library of Medicine), Web of Science (Clarivate Analytics) and Scopus (Elsevier). An update took place in June 2023. The searches did not have restrictions on date or language.

A search strategy was developed for each database using a combination of keywords, that included the following medical subject headings (MeSH) and free terms: (("Salivary Gland" OR "Salivary Gland Neoplasms" OR "Salivary Gland Neoplasm" OR "Cancer of the Salivary Gland" OR "Tumor of the salivary gland" OR "Tumor of the salivary gland" OR "Neoplasm of the salivary gland" OR "Salivary Gland Cancers" OR "Cancer of the Salivary Gland" OR "Salivary Gland Cancer" OR "Salivary Gland Tumor" OR "Salivary Gland Tumors" OR "Salivary Gland Tumor" OR "Salivary Gland Tumors" OR "Salivary Glands") AND ("Epigenetic alteration" OR "Epigenetic alterations" OR Epimutation OR "DNA Methylation" OR Hypermethylation OR Histone OR Histones OR "Histone alterations" OR "Histone modifications" OR Deacetylase OR microRNA OR miRNA OR miR OR "non-coding RNA"))

A grey literature search was performed on Google Scholar, OpenGrey and ProQuest. Also, a manual search on the references list from each included study was conducted with the aim to identify studies that may not have been detected through the electronic searches.

2.4. Inclusion and exclusion criteria

Cross-sectional studies, case-control studies, longitudinal studies, case series or case reports assessing the epigenetic alterations in SGTs, as well as the prognosis and progression of the tumors were included in this study.

Exclusion criteria encompassed the following: reviews, letters, personal or expert opinions, meeting abstracts, in vitro or ex vivo studies and animal studies.

Studies were categorized based on the specific epigenetic alteration they investigated to facilitate the synthesis of information.

2.5. Selection and data collection process

Reference management was carried out using Rayyan QCRI web application (Qatar Computing Research Institute, Doha, Qatar). After manually removing duplicate references, title and abstracts were assessed by two independent reviewers (K·S·S.V. and M.S·N.). References which title/abstract were according to the eligibility criteria were selected for full-text reading. The same independent reviewers assessed the studies selected for full-text reading. Studies that met the eligibility criteria after full-text reading were included in this systematic review and meta-analysis. In cases where there was a disagreement between the two reviewers concerning the selection of the studies, a third examiner (V·F·B.) discussed with the two reviewers to reach a consensus.

Data extraction was performed by two independent authors (M.S.N. and K·S·S·V.) and the information was crosschecked to verify its accuracy. A third author (V·F·B) was consulted to decide which information was relevant to data extraction and to solve any disagreement between the reviewers. The following data were extracted and registered in an electronic table: name of author and year of publication, study design, sample size and participant's sex and age, tumor type (benign or malignant) and diagnosis, epigenetic alteration studied, method of evaluation of this epigenetic alteration, clinical outcomes assessed and the results regarding the clinical outcomes of the tumor.

2.6. Assessment of methodological quality

The methodological quality assessment of the selected studies was conducted using the Critical Appraisal Checklist recommended by the Joanna Briggs Institute of the University of Adelaide, for cross sectional and case-control studies [12]. The studies were evaluated according to the following criteria:

Cross sectional studies were evaluated for clear definition of inclusion criteria, description of the subjects and the study setting, valid measurement of the exposure, measurement criteria, identification of confounding factors, valid measurement of outcomes and appropriated

statistical analysis. Case control studies were evaluated for comparability between the groups, pairing of cases and controls, criteria for identifying cases and controls, measurement of exposure, identification of confounding factors and strategies to deal with them, measurement of outcomes, length of exposure and appropriate statistical analysis.

The included studies were rated as "low risk of bias", "high risk of bias" or "unclear risk of bias", for each parameter. The assessment of methodological quality was conducted by two authors (K·S·S·V and M. S·N) independently and any disagreements between them were consulted to a third examiner (V·F·B.).

2.7. Statistical analysis, synthesis of the results and certainty of evidence

A descriptive analysis of the main findings from the included studies was performed, considering their main outcomes. This analysis synthesized the studies according to the specific epigenetic alterations studied and the types of tumors investigated. Meta-analysis was conducted with the included studies that exhibited methodological homogeneity. The software Review Manager 5.3 (Review Manager (RevMan) [Computer program], version 5.3; Copenhagen: The Nordic Cochrane Center, The Cochrane Collaboration, 2014) was used. Statistical heterogeneity was assessed by means of the I^2 statistics. The fixed model was deployed [13]. For the outcome of interest, Odds Ratio and confidence interval was used in the synthesis and presentation of the results.

The evaluation of the certainty of evidence was performed using the tool Grading of Recommendations, Assessment, Development and Evaluations (GRADE). According to GRADE, the certainty of evidence could be "very low", "low", "moderate" or "high" [14]. This assessment was made using the tool GRADEpro online [15].

3. Results

3.1. Study selection

The search comprised a total of 1807 studies. A total of 1344 records were screened for title and abstract according to the inclusion criteria, after the removal of 463 duplicates. A hundred and three studies were selected for full-text assessment. After the evaluation of the full text, 47 studies were included in this systematic review. The Grey Literature and manual search comprised 286 studies. After the assessment of title and abstracts, none of these references met the eligibility criteria. The PRISMA flowchart showing the selection process is outlined in Fig. 1. A list with the excluded studies after the evaluation of the full text is presented on the Supplementary File 1 along with the reason for exclusion of each paper.

3.2. Study characteristics

The 47 included studies and their general characteristics are described on Table 1. All included studies were published in English, between the years 2003 and 2023. Twenty-one studies were case-control, and 26 were cross-sectional studies. A total of 2768 tumor samples were identified in the 47 studies. The sample size in each study varied from 5 to 200 individuals. All studies included samples comprising SGTs, benign or malignant.

From the 47 studies, ten studies evaluated samples consisting in malignant and benign tumors [16–25]. Only one study evaluated samples from a benign tumor: pleomorphic adenoma (PA) [26]. Thirty-six studies evaluated samples only from malignant tumors: mucoepidermoid carcinoma (MEC), cystic adenocarcinoma, carcinoma ex pleomorphic adenoma, adenoid cystic carcinoma (ACC), carcinoma of the parotid gland, epithelial myoepithelial carcinoma, salivary duct carcinoma, carcinosarcoma, adenosquamous carcinoma, acinic cell carcinoma, basal cell adenocarcinoma [9,27–61]. In general, the studies evaluated aspects related to the methylation of specific genes, the occurrence of various types of miRNA, histones, and alterations in the expression of proteins with epigenetic significance.

3.3. Methodological quality of the included studies

The studies included presented a high overall risk of bias as shown on Fig. 2. For cross-sectional studies, the two criteria with the lowest risk of bias were the "measurement of the outcome in a valid and reliable way" and "measurement of the exposure in a valid and reliable way". The criteria with the highest risk of bias on the cross-sectional studies was the criteria about "strategies to deal with the confounding factors" (Fig. 2a). For case-control studies, three criteria had low risk of bias: evaluation of the measurement of exposure, the exposure period and the

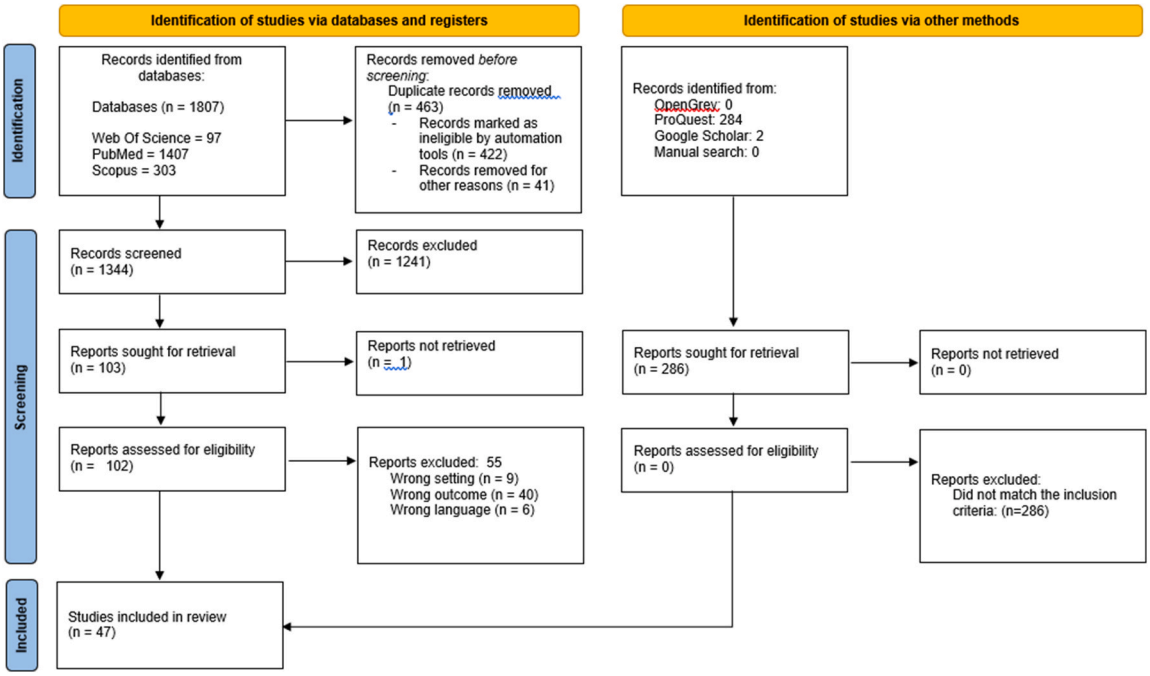

Fig. 1. Flow diagram showing the results of the search.

Table 1
Articles included in this systematic review and general characteristics of the studies.

(A)

Histones

Author, year	Sample size and participants sex and age	Type of tumor (benign or malignant) and diagnosis	Epigenetic alteration studied and method	Outcomes studied	Results of the association between epigenetic alterations and outcome
Lam-Ubol et al., 2022	70 cases Sex and age not mentioned.	**Malignant:** 30 MEC 20 ACC **Benign:** 20 PA	Histone H3 IHC	Perineural, vascular or bone invasions and tumor front invasion.	Upregulation of H3K9Me3 in MEC cases showing small nest invasion at the tumor front ($p = 0.017$) and in the advanced pathologic grades ($p = 0.028$).
Pouloudi et al., 2021	58 cases 18 males 40 females Mean age at diagnosis: Benign 57.72 years (ranging from 28 to 85 years) and Malignant: 71.14 years (ranging from 41 to 93 years).	**Benign:** 28 PA, 7 Warthin tumors, 1 Basal cell adenoma **Malignant:** 3 MEC, 4 ACC, 5 Acinic cell carcinomas, 1 Basal cell adenocarcinoma, 1 Salivary duct carcinoma, 1 Epithelial-myoepithelial carcinoma, 7 Squamous cell carcinomas	Histone deacetylase - HDAC-1, -2, -4 and -6 IHC	Overall survival, grade	HDAC-2 positivity was significantly associated with more prolonged overall survival (OS) of patients with malignant SGTs ($p = 0.028$). No HDAC-6 expression was significantly associated with prolonged OS of these patients ($p = 0.043$)
Wagner et al., 2017	84 cases Age at the time of diagnosis of 50.82 (±18.08) and a male: female ratio of 1:1.10.	**Benign:** 33 PA, 9 Warthin's tumor and **Malignant:** 22 ACC, 15 MEC, 5 acinic cell carcinoma	Histone modifications acetyl-histone H3 (lys9) expression IHC	Glandular location, stage	In advanced cases (III/IV), 66.7 % of patients presented hypoacetylated H3 (Lys9) lesions, compared with 50 % in the early cases (I/II). Despite the slight tendency observed, no significant difference was observed ($P = 0.36$, Fisher's exact test).
Xia et al., 2013	66 cases 36 males 30 females Age: from 16 to 82 years (mean, 53.02 years)	**Malignant:** ACC	Histones - H3K9me3 and H3K9Ac expression IHC	Glandular location, grade, perineural invasion, tumor's size, nodal metastasis, distant metastasis, recurrence, overall survival, disease-free survival	H3K9me3 expression was positively correlated with solid pattern tumors ($P = 0.002$) and distant metastasis ($P = 0.001$). There was no statistically significant correlation between the expression level of H3K9me3 or H3K9Ac and other clinicopathologic parameters. Survival rates: High levels of H3K9me3 showed significantly poorer OS outcomes than those with low levels of H3K9me3 ($P = 0.001$); patients with high H3K9Ac expression had a significantly better OS than those with low expression levels ($P = 0.05$)

(B)

miRNA

Author, year	Sample size and participants sex and age	Type of tumor (benign or malignant) and diagnosis	Epigenetic alteration studied and method	Outcomes studied	Results of the association between epigenetic alterations and outcome
Abdolrahmani et al., 2023	19 cases 5 controls Mean age: 47.63 ± 21.10 13 females 16 males	**Malignant:** MEC	miRNA miR-145 e miR21 Real Time PCR	Tumor size, local recurrence, grade and stage.	A positive association was found between miR-21 expression level and both histologic grade ($p = 0.004$) and tumor stage of MEC patients ($p = 0.004$). No significant association was found between miR-145 or miR-121 expression levels and tumor size and local recurrence.
Andreasen et al., 2018a	11 cases 7 controls 6 males 5 females Age not mentioned	**Malignant:** ACC	miRNA PCR	Glandular location, distant metastasis, grade	Comparing primary ACCs and metastasis as groups, there were no significantly differentially expressed miRNAs.
Andreasen et al., 2018b	184 cases Age and sex not mentioned	**Malignant:** ACC	miRNA PCR	Overall survival, recurrence free survival, stage	No miRNA was associated with overall survival, but high levels of hsa-miR-6835-3p were associated with reduced recurrence free survival ($p = 0.016$). There were no differentially expressed miRNAs between solid and tubulo cribriform ACCs or between high-stage (stage III + IV) and low-stage (stage I + II) disease at diagnosis.

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Table 1 (*continued*)

(B) miRNA					
Author, year	Sample size and participants sex and age	Type of tumor (benign or malignant) and diagnosis	Epigenetic alteration studied and method	Outcomes studied	Results of the association between epigenetic alterations and outcome
Bayat et al., 2023	15 cases 15 controls Cases: Age 56.07 ± 13.52. 9 male and 6 female Controls: age 56.40 ± 12.61. 8 male and 7 female.	**Malignant:** ACC	miRNA (miR-29, miR-205 and miR-93) RT-PCR	Tumor size, histopathologic grades, tumor stage, lymphovascular invasion. Perineural invasion	A significant difference was observed between ACC patients with and without perineural invasion regarding the median relative expression level of miR-29a ($p = 0.01$). There was a significant correlation between the relative expression level of miR-93-5p and histopathologic grade ($r = 0.65$ and $p = 0.01$).
Boštjančič et al., 2017	70 cases (39 men and 31 woman) 11 control (6 women and 4 men) Age not mentioned	**Malignant:** Carcinoma of the parotid gland, acinic cell carcinoma, MEC, carcinoma ex PA, ACC, poorly differentiated carcinoma, epithelial myoepithelial carcinoma, adenocarcinoma NOS, salivary duct carcinoma, small cell carcinoma, carcinosarcoma, adenosquamous carcinoma	MiRNA (miR-99b, miR 140 and let-7a) PCR	Overall survival, nodal metastasis, tumor's size	Comparison of favorable versus poor clinical prognosis group yields no statistically significant difference in expression of miRNAs. Comparing poor clinical prognosis and NSG, there were two differentially expressed miRNAs, miR-133b and miR-99b, which were both upregulated
Chen et al., 2023	25 cases 10 controls	**Malignant:** ACC	miRNA (miR—183-5p e miR-182-3p associated to FAT1 and YAP1) qRT-PCR	Distant metastasis	Lower expression of FAT1 and higher expression of yap1 at the leading edge were both closely related to higher rates of distant metastasis ($P = 0.036$ for FAT1, $P = 0.014$ for YAP1). The downregulation of FAT1 were mediated by dysregulates miRNAs: there were 58 upregulated miRNAs and 16 downregulated miRNAs in GSE59700 and upregulated miRNAs and 89 downregulated miRNAs in GSE117275 with stringent filtering criteria ($\|log2\text{-fold change}\| > 1$, FDR <0.05). miR-183-5p and miR-182-3p were upregulated in AAC tissues by 6.71 fold and 3.99-fold respectively, while only miR-183-5p expression was both inversely correlated with FAT1 expression and positively correlated with YAP1 expression.
Fu et al., 2020	52 cases 38 controls 26 males 26 females Age: 31–75 years	**Malignant:** ACC	miRNA (miR-103a-3p) RT-qPCR	Local recurrence, nodal metastasis, distant metastasis, tumor's size, perineural invasion, glandular location, grade.	High miR-103a-3p expression was associated with the local regional recurrence and lung metastasis. However, no significant associated was identified between miR-103a-3p expression and the other clinicopathological type.
Kerche et al., 2022	55 cases 10 controls Age and sex not mentioned.	**Benign:** 23 pleomorphic adenomas **Malignant:** 14 mucoepidermoid carcinomas, 18 adenoid cystic carcinomas	Expression of miR-9, miR-34a, miR-101, miR-138, miR-155 and miR-200c. PCR	Grade, metastasis, overall survival rate.	Increased expression of miR-155 in MECs was associated with low grade tumors ($p = 0.032$), whereas increased miR-200c expression was associated with the presence of MEC lymph node metastasis ($p = 0.018$). Patients with MECs exhibiting increased expression of miR34a ($p = 0.005$) and miR-138 ($p = 0.003$) had better overall survival than those with decreased non-altered expression. Poor overall survival rates were observed for patients with ACC and decreased expression of miR-9 and increased expression of miR-155 ($p = 0.029$ and $p = 0.007$).
Koparal et al., 2022	34 cases 34 controls Mean age 51.20 ± 20.14. 16 females 18 males	**Malignant:** 7 mucoepidermoid carcinoma **Benign:** 17 pleomorphic adenomas, 10 Warthin's tumor. Control with the adjacent normal tissue.	miR-200a, miR-30c and miR-373 involved in Wnt/β-catenin qPCR	Perineural invasion and lymph node metastasis status.	Patients without perineural invasion had significantly higher levels of miR-373 in salivary gland tumors compared to normal tissues, and its expression was found to be higher in tumor tissues of patients with lymph node metastasis compared to normal tissues ($p = 0.0065$). Expression of miR-30c which is

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Table 1 (*continued*)

(B)

miRNA

Author, year	Sample size and participants sex and age	Type of tumor (benign or malignant) and diagnosis	Epigenetic alteration studied and method	Outcomes studied	Results of the association between epigenetic alterations and outcome
					downregulated in malignant salivary gland tumors, was found to be downregulated in tumor tissues compared to normal tissues of patients without perineural invasion ($p = 0.0005$).
Liang et al., 2017	106 cases 20 controls Age and sex not mentioned	**Malignant:** ACC	MiR-125a-5p In situ hybridization and IHC	Nodal metastasis, distant metastasis, survival status, stage	miR-125a-5p expression was associated with nodal metastasis ($p = 0.039$), distant metastasis ($p = 0.032$), survival status ($p = 0.0066$), but not stage ($p = 0.458$).
Mitani et al., 2013	30 cases 4 controls Age and sex not mentioned	**Malignant:** ACC	miR-17-92 miRNA array platform	Tumor size, nodal metastasis, stage, and recurrence	Correlation of hsa-let-7a and size, stage and recurrence were statistically significant ($p = 0.012$, $p = 0.027$, $p = 0.04$, respectively). Correlation of hsa-miR-150 and nodal metastasis and stage was statistically significant ($p = 0.019$, $p = 0.013$, respectively).
Xie et al., 2018	102 cases Age and sex not mentioned	**Malignant:** ACC	ADAMTS9-AS2 expression, miR-2392, miR-362-5p, miR-193a-5p, miR-143-3p, miR-493-5p qRT-PCR	Tumor's size, stage, distant metastasis, overall survival	Reduced ADAMTS9-AS2 expression in ACC patients was closely associated with tumor size ($p = 0.017$), clinical stage ($p = 0.033$), and distant metastasis ($p = 0.035$). Kaplan-Meier analysis indicated that increased ADAMTS9-AS2 expression in ACC was significantly associated with decreased overall survival. Through qRT-PCR analysis, we found that the expression levels of miR-143-3p were significantly downregulated in ACC patients with metastasis
Zhang et al., 2021	158 cases 85 males 73 females 73 patients <50 years old and 85 patients ≥50 years old.	**Malignant:** ACC	miRNA (miR-187) FISH (fluorescent *in situ* hybridization)	Perineural invasion	miR-187 presented a downward trend at the nerve invasion frontier.
Zhu et al., 2021	36 cases Sex and age not mentioned.	**Malignant:** ACC	miR-375, miR-494, miR-34c-5p and miR-331-3p. Reverse transcription-quantitative PCR (RT-Qpcr)	Clinical stage, living status and overall survival.	Only miR-331-3p appeared to influence the patient's overall survival.

(C)

Methylation

Author, year	Sample size and participants sex and age	Type of tumor (benign or malignant) and diagnosis	Epigenetic alteration studied and method	Outcomes studied	Results of the association between epigenetic alterations and outcome
Agnese et al., 2006	28 benign 5 malignant Age and sex not mentioned	**Benign:** PA **Malignant:** cystic adenocarcinoma, carcinoma ex PA	Methylation status of the p16INK4a gene promoter Methylation-specific PCR	Glandular location, grade, tumor's size, stage, nodal metastasis	No significant association was observed between p16 hypermethylation and clinicopathological variables in all the tissue samples analyzed.
Bell et al., 2011	16 cases 16 controls 7 males 9 females Mean age: 57.6 years	**Malignant:** ACC	Hypermethylation of CpG islands Amplification and microarray method and the pyrosequencing technique	Tumor size, perineural invasion, glandular location	Comparing the methylation status of the 32 genome loci with the clinical and pathological parameters of the patients yielded the EN1 hypermethylated gene promoter as the best fit. No stronger correlations with clinical and pathological parameters were noticed for the rest of the hyper- and hypomethylated genes.
Ge et al., 2011 (Human Pathology)	41 cases 41 controls 24 males 17 females	**Malignant:** ACC	Methylation spreading pattern of runt-related transcription factor-3 (RUNX3) C-phosphate-G (CpG) island (3478 base pairs)	Perineural invasion, glandular location, grade, nodal metastasis, distant	No significant correlation was found between RUNX3 methylation levels and any clinicopathologic parameter.

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Table 1 (*continued*)

(C)

Methylation

Author, year	Sample size and participants sex and age	Type of tumor (benign or malignant) and diagnosis	Epigenetic alteration studied and method	Outcomes studied	Results of the association between epigenetic alterations and outcome
	Age ranged from 34 to 81 years (median age, 57 years)		methylation RT-PCR	metastasis, tumor's size.	Relationships between RUNX3 methylation levels are summarized in Table 2.
Ge et al., 2011 (Oncology Reports)	114 cases 54 males 60 females Age: 23–81 years (median age 55 years)	**Malignant:** ACC	Hypermethylation of RUNX3 RT-PCR	Perineural invasion, nodal metastasis, stage, distant metastasis, grade, glandular location	There was a significant correlation between RUNX3 methylation and perineural invasion, nodal metastasis, stage and distant metastasis ($p < 0.001$). There was no significant correlation between the degree of RUNX3 methylation and glandular location and histologic types of ACCs (all $P > 0.05$)
Guo et al., 2007	36 cases 6 controls Age and sex not mentioned	**Malignant:** MEC	Hypermethylation of the p16ink4a Methylation specific PCR	Stage, grade	There was no statistical difference according to tumor stage or histological grade ($P > 0.05$) (Table 2)
Hu et al., 2011	50 cases 14 females 36 males Age: median age of 55 (from 34 to 78)	**Malignant:** Carcinoma ex pleomorphic adenoma	Methylation of p16 IHC and PCR	Glandular location, stage, grade	No associations were found between promoter methylation of the p16 gene and glandular location ($p = 0.69$), TNM stage ($p = 0.96$), grade ($p > 0.99$) Table 3
Kishi et al., 2005	36 cases Age and sex not mentioned	**Malignant:** 17 ACC, 7 MEC, 3 squamous cell carcinomas, 3 acinic cell carcinomas, 2 carcinomas in PA, 2 adenocarcinomas, 1 salivary duct carcinoma, 1 basal cell adenocarcinoma	Methylation in INK4a/ARF, RB1, p21, p27, PTEN, p53, MDM2 and O6-MGMT PCR	Glandular location, stage, overall survival	Patients with methylated RB1 had a significantly shorter survival compared to those without methylation ($p = 0.02$). Correlation between epigenetic alterations and clinical parameters were not detected.
Lee et al., 2008	69 cases 39 males 30 females Age ranged from 8 to 89 years, with a mean age of 55.7 years.	**Malignant:** 13 acinic cell, 25 ACC, 17 MEC, 8 salivary duct carcinomas, 6 carcinoma ex PA	Hypermethylation status of the retinoid acid receptor h2 (RARb2), RAS association domain family 1A (RASSF1A), O6-methylguanine-DNA methyltransferase (MGMT), and E-cadherin genes Quantitative pyrosequencing and qualitative methylation-specific PCR	Tumor's size, grade, glandular location, nodal metastasis, 3-year survival	There were no statistically significant correlations between methylation status and glandular location and stage. There was a significant correlation between RARb2 methylation status by pyrosequencing and tumor grade ($P = 0.014$), tumor size ($P = 0.008$), 3-year survival ($P = 0.002$), and 5-year survival ($P = 0.028$).
Lee et al., 2010	46 cases 25 controls Age and sex not mentioned	**Malignant:** MEC	Methylation status of APC PCR	Glandular location, grade, stage	There is no significant correlation between APC, SFRP gene methylation, and clinicopathologic features of MEC
Li et al., 2005	60 cases 28 females 32 males Median age of 53 years (range, 16–71 years).	**Malignant:** ACC	Promoter methylation of p16ink4a, RASSF1A and DAPK Methylation specific PCR	Grade, stage, perineural invasion, nodal metastasis, distant metastasis, glandular location	RASSF1A promoter methylation was more frequent in high-grade tumors than in low-grade tumors ($P = 0.009$), in advanced stage tumors ($P = 0.008$), and in tumors with metastasis ($P = 0.005$). Among 9/25 tumors that exhibited RASSF1A promoter methylation had either lymph node or distant metastasis, compared with only 2/35 tumors without RASSF1A promoter methylation (Table 1) ($P = 0.005$). Methylation status was not associated with neural invasion by tumors ($P = 1.0$). The frequencies of promoter methylation were similar between major gland tumors and minor gland tumors, with the exception of MGMT promoter methylation.
Martinez-Marcial et al., 2022	5 cases All cases female	**Malignant:** MEC	Methylation of the genes hMLH-1 and P16 PCR	Grade	There are no trend or statistical significance in the methylation of

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Table 1 (*continued*)

(C)

Methylation

Author, year	Sample size and participants sex and age	Type of tumor (benign or malignant) and diagnosis	Epigenetic alteration studied and method	Outcomes studied	Results of the association between epigenetic alterations and outcome
	Mean age of 52 ± 18.6 years old.				hMLH-1 and P16 and grade or severity.
Maruya et al., 2003	32 cases Age and sex not mentioned	**Malignant:** ACC	Hypermethylation of p16 promoter Methylation specific PCR	Grade, glandular location	There was no significant correlation between the p16 methylation status and histological grade and glandular location.
Maruya et al., 2004	23 cases Age and sex not mentioned	**Malignant:** ACC	Hypermethylation of the promoter of e-caderin Methylation specific PCR.	Grade, glandular location, death	Promoter methylation had no correlation with the histological grade or patient prognosis.
Nikolic et al., 2015	60 cases 77 controls Age and sex not mentioned	**Benign:** PA (50) **Malignant:** Carcinoma ex PA (10)	P16 and p14 promoter hypermethylation PCR	Tumor's size	In PA group, methylation was not related to clinicopathological features (Table 2). In CXPA group hypermethylation of the two genes was more frequent in advanced clinical stages (small number of patients, the statistical analysis in unreliable).
Nikolic et al., 2018	35 cases 10 controls Age and sex not mentioned	**Malignant:** MEC	P14 methylation PCR	Stage, survival rates	All MEC cases harboring TP53 hypermethylation had been classified as lower clinical stages ($P = 0.033$). Similarly, hTERT hypermethylation dominated in stages 1&2 compared to stages 3&4 ($P = 0.002$). There was no association between methylation and histological grades. Promoter methylation status did not show statistically significant association with survival rates, although patients with methylated p16 promoter appeared to have poorer survival than those with unmethylated p16. On the contrary, patients with methylated TP53 and hTERT promoter had a trend of better overall survival compared to patients with unmethylated TP53 and hTERT ($P = 0.120$ and $P = 0.151$, respectively).
Pereira et al., 2017	23 cases 12 controls Mean age of 34.8 years (70 ± 11)	**Benign:** PA	Methylation profile in 22 apoptosis-related genes DNA methylation PCR array	Glandular location, tumor's size, recurrence	Only tumor's size was evaluated. BCL2L11 showed marked difference in methylation levels for normal glands and tumors, with methylation percentage of 75.4 % in PA > 2 cm, 85.5 % in PA ≤ 2 cm, while showing 17.9 % of methylation in NSG.
Scesnaite et al., 2014	36 cases Age and sex were not described	**Malignant:** 36 patients with salivary gland carcinoma (not specified) 19 histologically matched normal tissues	O-6-methylguanine-DNA methyltransferase (MGMT) methylation Quantitative pyrosequencing	Tumor's size, glandular location, nodal metastasis, distant metastasis, stage, grade, death	The methylation status in tumors did not correlate with any clinicopathologic and demographic variables.
Shao et al., 2011	18 cases 13 controls	**Malignant:** ACC	De-methylation AQP1, CECR1, C1QR1, CTAG2, P53AIP1, TDRD12, BEX1, and DYNLT3 Quantitative methylation-specific PCR (qMSP)	Margin status, perineural invasion, glandular location, stage, overall survival, recurrence and distant metastasis	AQP1 hypomethylation did not correlate with margin status, perineural invasion, or stage. AQP1 methylation status was not associated with differences in overall survival. Because of the small sample size and relatively few numbers of events, it was not statistically viable to draw accurate conclusions for the risk of locoregional recurrence or the development of distant metastasis.
Sirivanichsuntorn et al., 2013	24 cases 14 controls 10 males 14 females	**Malignant:** MEC	LINE 1 and ALU hypomethylation PCR	Grade	The reduced methylation levels of LINE-1 were correlated with a poorer histological grade

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Table 1 (*continued*)

(C)

Methylation

Author, year	Sample size and participants sex and age	Type of tumor (benign or malignant) and diagnosis	Epigenetic alteration studied and method	Outcomes studied	Results of the association between epigenetic alterations and outcome
	Mean age ±SD = 39.62 ± 12.37 years				
Tan et al., 2014	77 cases 30 controls 49 males 58 females	**Malignant:** ACC	Aquaporin 1 promother hypermethylation Quantitative methylation-specific PCR (qMSP)	Glandular location, stage, nodal metastasis, distant metastasis, grade, perineural invasion, overall survival	AQP1 promoter methylation levels were not correlated with glandular location, stage, nodal metastasis, distant metastasis, grade, perineural invasion. Univariate analysis revealed that AQP1 hypermethylation was associated with increased overall survival.
Uchida et al., 2004	24 cases 10 controls Age and sex not mentioned	**Malignant:** ACC and MEC	Hypermethylation of 14-3-3 s gene Methylation specific PCR	Glandular location	The study showed descriptive results to methylations status and glandular location (Table 1)
Wang et al., 2015	14 cases 14 controls Age and sex were not mentioned	**Malignant:** MEC	Methylation of the CLIC3 Illumina Human Methylation27 Bead Chip array and differential methylation analysis - Quantitative methylation-specific PCR	Grade, stage, glandular location, margin status, perineural invasion	There were no significant differences in the level of CLIC3 methylation by clinical or pathological characteristics (Table 2 showed detailed analysis)
Williams et al., 2006	102 cases Age: 8–90 years (mean, 56.9 years) Benign: not available	**Benign:** 2 myoepitheliomas, 12 PA and 9 Warthin's tumors. **Malignant:** 14 acinic cell carcinomas, 26 ACC, 18 MEC, and 21 salivary duct carcinomas	Methylation of RASSF1, RARb2, DAPK, and MGMT Methylation specific PCR	Glandular location, tumor's size, nodal metastasis, distant metastasis, death	High-grade phenotypes (dedifferentiated acinic cell carcinomas, solid or dedifferentiated ACC, high-grade MEC and SDC) were more often methylated than low-grade phenotypes (21 of 35 versus 12 of 32 respectively; P = 0.006) Although no significant statistical correlation was found between methylation pattern and tumor metastasis, 52.4 % (22 of 42) of metastatic tumors showed methylation of at least one gene compared with 29.7 % (11 of 37) of nonmetastatic tumors that showed methylation (P = 0.07).
Zhang et al., 2014	167 cases 50 controls 78 females 89 males Age: 52.5 years (range 27–83).	**Malignant:** ACC	RASSF1A promoter hypermethilation Bisulfite sequencing PCR (BSP) and/or methylation-specific PCR (MSP)	Grade, stage, survival	RASSF1A promoter hypermethylation was detected in 35.3 % (59/167) of ACC tissues and was associated with histologically solid tumor pattern (P = 0.002), advanced TNM stage (P = 0.014), poor over-all survival (Log-rank test, P = 0.001), disease-free survival (Log-rank test, P,0.001) and identified as an independent predicator of over-all patient survival (P = 0.009) and disease-free survival (P = 0.001)

(D)

Other epigenetic alterations

Author, year	Sample size and participants sex and age	Type of tumor (benign or malignant) and diagnosis	Epigenetic alteration studied and method	Outcomes studied	Results of the association between epigenetic alterations and outcome
Andisheh-Tabir et al., 2016	20 benign 36 malignant 23 controls 18 males 38 females Mean age: 48.36 ± 11.3	**Benign:** PA **Malignant:** MEC, ACC	Metastasis-associated genes 1 (MTA1) Immunohistochemistry (IHQ)	Tumor size, nodal metastasis, distant metastasis, grade and stage	No statistically significant correlation between MTA1 protein expression and any clinicopathological features (P > 0.05).
Bell et al., 2012	200 cases 99 males 101 females	**Malignant:** ACC	EN1 protein expression IHQ	Overall survival	Epithelial EN1 positivity in patients with ACC was correlated significantly with poor survival (P = 0.014).

(*continued on next page*)

Table 1 (*continued*)

(D)					
Other epigenetic alterations					
Author, year	Sample size and participants sex and age	Type of tumor (benign or malignant) and diagnosis	Epigenetic alteration studied and method	Outcomes studied	Results of the association between epigenetic alterations and outcome
	Age: Ranged from 15 to 86 years, median age 52.				
Chiosea et al., 2008	78 MEC 42 females 36 males Median age (range): 51.5 (7–81)	**Malignant:** MEC	Dicer IHQ	Nodal metastasis, grade, stage, margin status, glandular location, recurrence	Dicer over- and/or under-expression were more commonly seen in high-grade MEC (83 %) than in low/intermediate grade MEC (35 %; p = 0.002) and in stage III/IV MEC (80 %) than in stage I/II MEC (41 %; p = 0.04).
Dai et al., 2014	135 cases 135 controls 85 males 50 females Age was not detailed	**Malignant:** ACC	Ubiquitin-Specific Peptidase 22 (USP22) IHQ	Grade, nodal metastasis, tumor's size, perineural invasion	High expression of USP22 was significantly correlated with histological subtype, lymph node metastasis, grade (p = 0.041, p = 0.001, p = 0.013, respectively)
Hajosi-Kalcakosz et al., 2015	94 cases Age and sex not mentioned	**Malignant:**17 MEC, 13 ACC, 8 carcinoma ex pleiomorphic adenoma, 5 acinic cell carcinoma, 3 Polymorphous low grade adenocarcinoma 8 others **Benign:** 18 PA, 13 Warthin tumor and 9 others	Enhancer of zeste homologue 2 (EZH2) IHC	Grade, perineural invasion, nodal metastasis	Descriptive analysis: No reliable relationship could be observed with tumor grade, but poorly differentiated components with infiltrative growth pattern were also positive. Perineural invading components of the tumors were also positive. The nodal metastasis of a poorly differentiated adecarcinoma was also positive.

MEC: mucoepidermoid carcinoma; PA: pleomorphic adenoma; ACC: adenoid cystic carcinoma; IHC: immunohistochemistry.

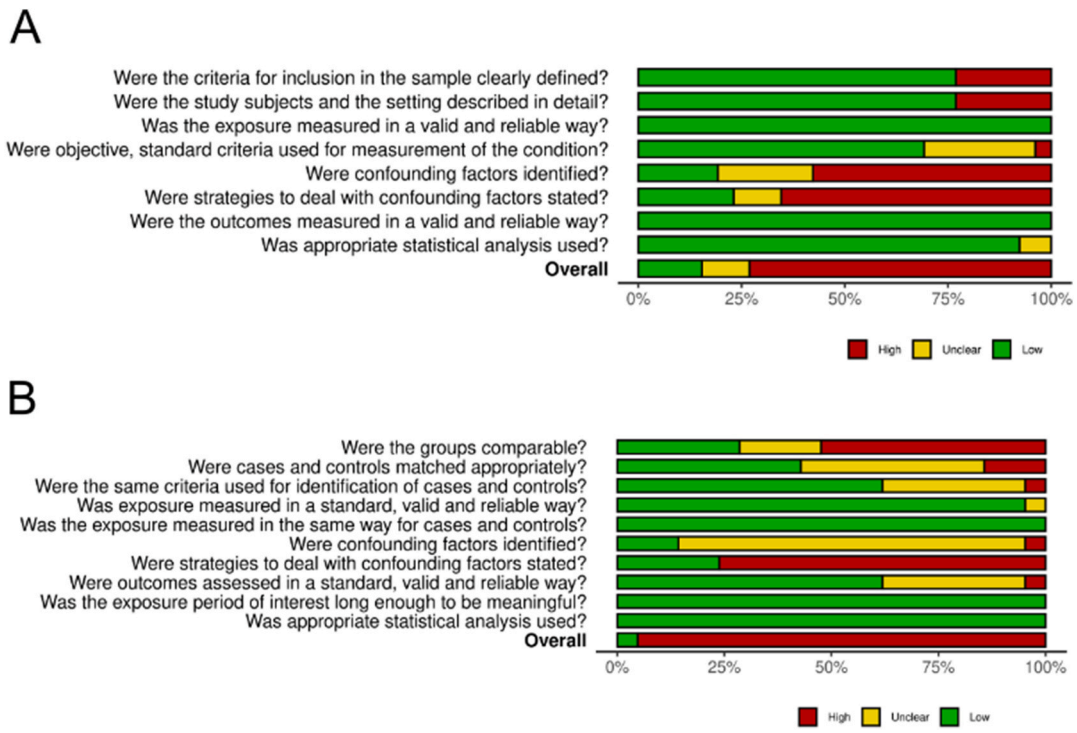

Fig. 2. Summary plot of the critical appraisal checklist (Joanna Briggs Institute) for the included studies. **A:** Summary critical appraisal for cross-sectional studies. **B:** Summary critical appraisal for case-control studies.
Created with RobVis (https://www.riskofbias.info/welcome/robvis-visualization-tool).

appropriate statistical analysis. The criteria with the highest risk of bias were "strategies to deal with the confounding factors" (Fig. 2b). A traffic light plot showing the appraisal of methodological quality for individual cross-sectional and case-control studies is shown on Supplementary Files 2 and 3 respectively.

3.4. Results of the individual studies

3.4.1. Histones

Only four studies in this systematic review assessed histones [19,20,22,27], and all of them were cross-sectional studies. These studies differed in terms of the outcome they evaluated. The outcomes assessed

were perineural/vascular invasion [19,27], survival [20,27], grade [20,27], stage [22], metastasis [27], recurrence [27] and tumor size [27]. The specific gene associated with histones was the heterogenous point among these studies, making it impossible to compare them in a meta-analysis. The studies evaluated the following genes: Histone H3 [19], HDAC-1 [20], HDAC-2 [20], HDAC-3 [20], HDAC-5 [20], HDAC-6 [20], H3K9me3 [27] and H3K9Ac [27]. Wagner et al., 2017, Pouloudi et al., 2021 and Xia et al., 2013 did not find any significant association between the expression of the histones studied and the outcomes evaluated. Lam-Ubol et al., 2022 showed that the upregulation of the H3K9me3 was associated to small nest invasion at tumor front ($p = 0.017$) and to advanced pathologic grades ($p = 0.028$) [19]. Detailed results for each study are shown on Table 1(A).

3.4.2. miRNA

Fourteen studies assessed the association between specific miRNAs and the clinical outcomes of SGTs [9,17,18,28–37,61]. Only four studies were designed as cross-sectional studies, while 10 studies were designed as case-controls. The assessed outcomes were grade [9,17,28,30,33], recurrence [9,28,33,35], metastasis [17,18,29,32,33,35,61], survival [9,17,31,37,61], stage [9,30,35,37,61], lymphovascular invasion [30] and perineural invasion [18,30,33,36]. There were two limiting factors regarding the expression of miRNA that made it unfeasible to conduct grouping analysis in this study. First, there were too many different miRNAs among the studies (miR-145, miR-21, miR-29, miR-205, miR-93, miR-99b, miR-140, miR—183-5p e miR-182-3p, miR-103a-3p, miR-9, miR-34a, miR-101, miR-138, miR-155, miR-200c, miR-200a, miR-30c, miR-373, MiR-125a-5p, miR-17-92, miR-2392, miR-362-5p, miR-193a-5p, miR-143-3p, miR-493-5p, miR-187, miR-375, miR-494, miR-34c-5p and miR-331-3p). The second factor is the absence of numerical data in papers concerning the association between miRNAs and the clinical outcomes of interest. Many studies brought clinical outcomes as a secondary outcome, showing the p value for the association without the absolute numbers. On these cases a contact attempt with the corresponding author was made, without any return from authors of studies assessing miRNAs. The results of individual studies assessing miRNAs are shown on Table 1(B).

3.4.3. Methylation

Most of the studies included in this systematic review evaluated the methylation of specific genes and their potential association with clinical outcomes (24) [23–26,38–57]. There were numerous different genes and subgroup analysis was only possible for RASSF1A and MGMT (Fig. 3). In summary, the studies evaluated the genes $p16^{INK4a}$, CpG islands, RUNX3, RB1, p21, p27, PTEN, p53, MDM2, O6-MGMT, RARb2, RASSF1A, APC, DAPK, hMLH, P16, P14, AQP1, CECR1, C1QR1, CTAG2, P53AIP1, TDRD12, BEX1, DYNLT3, LINE1, ALU, CLIC3. Some studies did not show absolute numbers for the associations, and the authors were contacted with a request of data, without return. Results of individual studies were heterogenous, some showing association between methylation in a specific gene while others did not show any association. The results from each study are shown on Table 1(C).

3.4.4. Other epigenetic alterations

Five studies focused on epigenetic alterations other than miRNAs, histones and methylation (Table 1(D)). Andisheh-Tabir et al., 2016 evaluated the metastasis-associated genes 1 (MTA1) and found no statistically significant correlation between MTA1 protein expression and any clinicopathological features. Bell et al., 2012 evaluated the EN1 protein expression and showed that EN1 expression was correlated with poor survival [58]. Chiosea et al., 2008 evaluated the expression of Dicer and concluded that modifications on dicer expression were more common in high grade tumors [59]. Dai et al., 2014 assessed the Ubiquitin-Specific Peptidase 22 (USP22) and showed that high

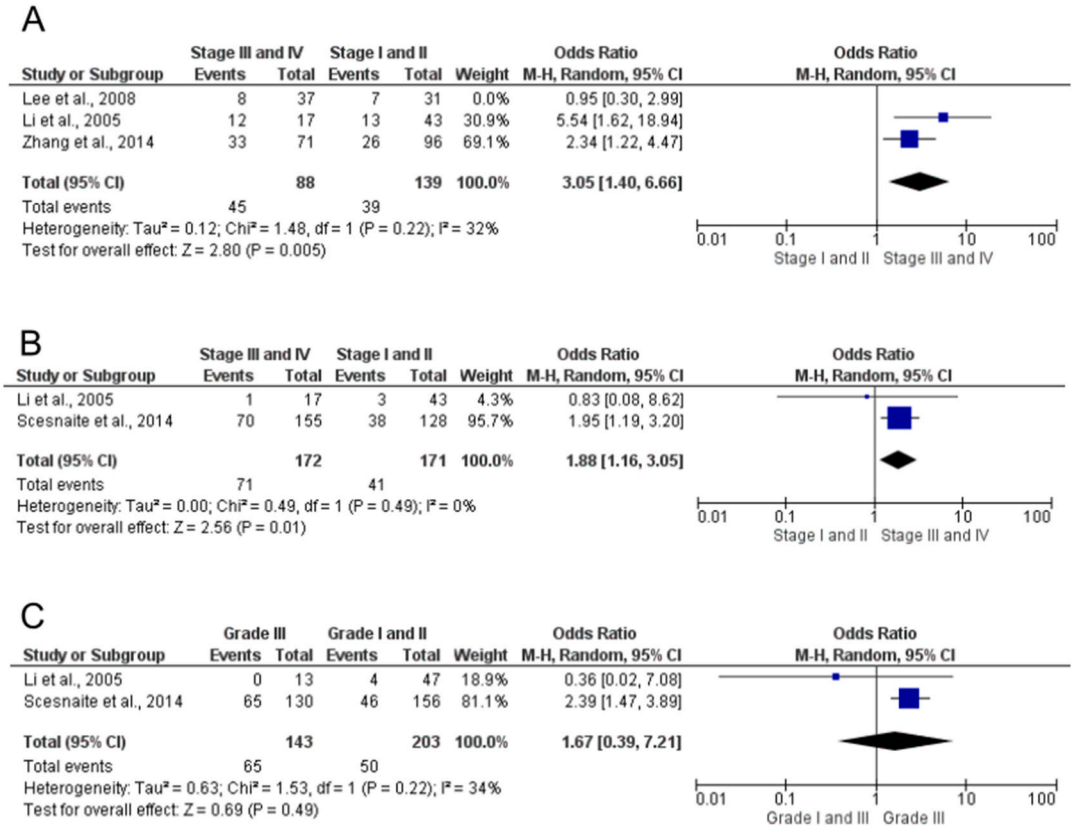

Fig. 3. Forest plot of the meta-analysis for the studies evaluating the methylation of the gene (A) RASSF1A comparing stages of ACC tumors and of the gene MGMT, comparing (B) stage and (C) grade for malignant tumors.

expression of USP22 was significantly correlated with histological subtype, lymph node metastasis and grade [60]. Hajosi-Kalcakosz et al., 2015 evaluated Enhancer of Zeste Homologue 2 (EZH2) expression and did not observe any relationship with tumor grade, but an association was described for perineural invading and metastasis [21].

3.4.5. Meta-analysis

The subgroup meta-analysis was possible for methylation, specifically for two genes, RASSF1A and MGMT, expressed in malignant tumors. A subgroup meta-analysis was not possible for benign tumors once there were only a few studies assessing them.

The comparison of stage (high/low) for ACC showed that RASSF1A methylation is related with higher stages ACC (OR: 3.05; 95 % CI: 1.40–6.66; $I^2 = 32$ %) (Fig. 3A).

For MGMT methylation, a comparison of stage and grade was possible gathering malignant tumors (ACC and salivary duct carcinoma). Meta-analysis shows that MGMT methylation on malignant tumors is associated with higher stages (III/IV) (OR: 1.88; 95 % CI: 1.16–3.05; $I^2 = 0$ %) (Fig. 3B). However, there is no significant difference when the same comparison was made for grade (OR: 1.67; 95 % CI: 0.39–7.21; $I^2 = 34$ %) (Fig. 3C).

3.4.6. Certainty of evidence

The assessment of the quality of evidence showed a very low certainty for all the subgroups analysis. The complete information is presented on Table 2.

4. Discussion

4.1. General aspects

Epigenetic alterations including miRNA, histone alterations, DNA methylation and some specific protein expressions are discussed in several types of tumors [62,63]. The presence of epigenetic alterations in salivary gland neoplasms has been also described and these studies indicated that these alterations play an important role in diagnosis and prognosis of tumors [5,9,21]. However, to our knowledge, none of these papers comprised all the epigenetic alterations described in the literature or included all benign and malign neoplasms. Herein we systematically reviewed and meta-analyzed the current literature to verify the existing evidence about the epigenetic alterations associated to SGTs and their possible role in the progression and prognosis of the tumor. Despite reviewing all epigenetic alterations, they were described in separated topics to make it more succinct.

4.2. Histones

Histones are a heterogeneous group of proteins responsible for organizing the nuclear architecture. Alterations in histones can regulate dynamic changes in chromatin architecture, which might lead to modification of the transcriptional activity, repair, and replication [20]. Herein we review four studies related to histones alteration in different mechanisms. Lam-Ubol et al. [19] observed that H3K9Me3 had a statistically significant association with advanced pathologic grades in MEC. Pouloudi et al. [20] investigated histone deacetylase (HDAC-1, -2, -4 and -6) and found a statistically significant association between HDAC-2 positivity and a more prolonged overall survival in malignant salivary gland neoplasms. Xia et al. [27] observed H3 methylation and acetylation and found that H3K9me3 was correlated with solid pattern tumors, distant metastasis, and poor overall survival rates in ACC. In the other hand, Wagner et al. [22] did not find any statistically significant alteration in H3. In light of these findings, we highlight the important role of histone acetylation, resulting in open or active chromatin structure and methylation resulting in some tumor suppressor gene silencing.

4.3. miRNA

MicroRNAs are short, non-coding RNA that function as potent post-transcriptional regulators of gene expression and modulate cellular activities such as tumor progression. In this study, we summarize fourteen studies that accessed the miRNAs relation to progression and prognosis of SGTs. However, it was not possible to perform meta-analysis due to the heterogeneity of the studies and individual results were displayed in Table 1. In a systematic review published in 2021, the prognostic value of miRNA in SGTs was described by dos Santos and colleagues [5] and the pooled HR was 2.35 (95 % CI, 1.77–3.10, $P < 0.00001$, $I2 = 76$ %), indicating that miRNA expression is an independent prognostic factor for shortened survival of patients with SGTs, more specifically ACC. In addition, the combined expression of some miRNAs was associated with several prognostic factors such as tumor size, distant metastasis, and shortened survival. In the present study, we observed statistically significant association between histologic grade, tumor stage, size, reduced recurrence free survival, perineural invasion, distant metastasis, lung metastasis, lymph node metastasis, and local regional recurrence. This compilation reinforces the importance of miRNAs in tumor progression and prognosis.

4.4. Methylation

DNA methylation affects normal DNA function, the interaction with proteins and the expression of specific genes. Gene methylation was the most common epigenetic alteration in this review and the methylation

Table 2
Assessment of the certainty of evidence (GRADE).

Variable	Certainty assessment							Number of individuals		Certainty
Outcome	Studies	Design	Bias	Inconsistency	Indirectness	Imprecision	Dose/response gradient	Low stage/ grade	High stage/ grade	
RASSF1A methylation (stage)	2	Observational Studies	Serious[a]	Not serious	Not serious	Serious[b]	Yes[f]	45/88 (51.13 %)	39/139 (28.05 %)	⨁◯◯◯ Very low
MGMT methylation (stage)	2	Observational Studies	Serious[a,c]	Serious[d]	Not serious	Serious[b]	Yes[f]	71/172 (41.27 %)	41/171 (23.97 %)	⨁◯◯◯ Very low
MGMT methylation (grade)	2	Observational Studies	Serious[a,c]	Serious[d]	Not serious	Serious[b,e]	Yes[f]	65/143 (45.45 %)	50/203 (24.63 %)	⨁◯◯◯ Very low

[a] Did not identify confounding factors neither used strategies to deal with them.
[b] Number of individuals is lower than the optimal information size.
[c] Did not define clearly the inclusion criteria for the sample.
[d] There were a wide variation of the estimative effect and a overlap of the confidence intervals.
[e] Confidence interval crossing the central axis.
[f] The intensity of expression is related to the tumor's worse behavior.

studies were included in meta-analysis. Methylation of CpG-enriched promoters is an important mechanism in the regulation of gene expression, in general this methylation often results in silencing tumor suppressor genes such as RASSF1A [64]. RASSF1A is a well described tumor-suppressor gene and the homologous protein. Dubois et al. [65] reviewed the role of RASSF1A in tumorigenesis and metastasis and discussed the importance in deregulation of cell proliferation, cell death, invasion, and to distant metastasis. Li et al. [46] concluded that RASSF1A promoter methylation was more frequent in high-grade ACC tumors than in low-grade tumors. Herein, meta-analysis also showed that RASSF1A methylation is related with higher stages of ACC.

4.5. Methodological quality evaluation

The quality assessment of the studies included in this systematic review and meta-analysis demonstrated that most studies was classified as low quality. From the twenty-one individual case-control studies only one research was low risk of bias [30] and from the twenty-six individual cross-sectional studies four demonstrated low risk of bias [38, 58–60], and three studies were classified as unclear risk of bias [19,27, 36].

5. Conclusion

This review showed the diversity of epigenetic changes demonstrated in studies. This reaffirms the importance of epigenetics in the progression and prognosis of salivary gland tumors. In summary, acetylation and methylation of histones have an important role in chromatin compaction and gene expression and most of the studies showed a significant association between histones alterations and worse prognosis. Regarding miRNAs, too many different miRNAs were discussed among the studies, and we observed divergent findings, most of which showing association with worse prognosis. As far as we know, there are no studies gathering information about all epigenetic alterations in salivary gland tumors. Even though we summarized a large number of studies and data, a great part of these studies could not be compared in a meta-analysis due to their methodological heterogeneity. Altogether, the methodological heterogeneity and the considerable number of studies classified with low methodological quality might be considered as a weakness of this study, and the strength of this study lies on the number of studies and data gathered, and also the comparison of some of the studies in meta-analysis. Finally, in this study we highlight the importance of understanding epigenetic changes and the prognosis and progression of salivary gland tumors.

CRediT authorship contribution statement

Karolina Skarlet Silva Viana: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Mariana Saturnino de Noronha:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. **Cristiane Squarize:** Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing. **Lucas Guimarães Abreu:** Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing. **Maria Cássia Ferreira Aguiar:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Vanessa Fátima Bernardes:** Conceptualization, Formal analysis, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.oor.2024.100176.

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