

SYSTEMATIC REVIEW

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Role of Cancer Associated Fibroblast (CAF) derived miRNAs on head and neck malignancies microenvironment: a systematic review

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Abstract

Background and aim MicroRNAs (miRNAs) play a key role in regulating gene expression within the tumor micro-environment, influencing cancer progression and therapy response. Cancer-associated fibroblasts (CAFs) contribute to tumor development by secreting exosomal miRNAs that promote proliferation, invasion, and resistance. This systematic review evaluates the impact of CAF-derived miRNAs on head and neck malignancies.

Methods A systematic search was conducted in PubMed, Scopus, WOS, and Google Scholar following PRISMA guidelines. Studies focusing on CAF-derived miRNAs in head and neck cancers were included. Data extraction covered study characteristics, miRNA profiling methods, functional roles, and clinical significance. The Scirap tool was used for quality assessment.

Results Among 921 identified articles, 21 met the inclusion criteria. Findings indicate that miR-21-5p, miR-106-5p, and miR-196a drive tumor progression in oral squamous cell carcinoma (OSCC), while miR-124 and miR-34a-5p act as suppressors. In esophageal squamous cell carcinoma (ESCC), miR-21 and miR-27a/b contribute to chemotherapy resistance, whereas miR-100-5p inhibits lymphangiogenesis. In head and neck squamous cell carcinoma (HNSCC), miR-196a and miR-196b may serve as diagnostic biomarkers. Exosomal miR-106a-5p promotes nasopharyngeal carcinoma (NPC) metastasis, and miR-7 and miR-196a contribute to therapy resistance in head and neck cancer (HNC).

Conclusion CAF-derived miRNAs significantly influence tumor progression, metastasis, and therapy resistance. These findings highlight their potential as biomarkers and therapeutic targets, warranting further clinical research for personalized treatment strategies.

Keywords CAF, Tumor microenvironment, Cancer-associated fibroblast, MiRNA, Head and neck malignancy

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Introduction

Cancer-associated fibroblasts (CAFs) are the primary stromal cells in the microenvironment, characterized by their spindle-shaped morphology and the presence of α -smooth muscle actin (α -SMA) [1]. Cancer-associated fibroblasts (CAFs) are a prominent element in the supportive tissue of tumors and play a stimulating function in several types of cancer, such as breast, prostate, and oral cancers [2]. Moreover, CAFs are frequently linked with high-grade malignancies and unfavorable prognoses. [1]. Cancer-associated fibroblasts (CAFs) are a prevalent element of the tumor microenvironment (TME) that promotes tumor growth. In terms of their physical presence, they can form a barrier that protects tumor cells from immune responses and various medications. [3]. Cancer-associated fibroblasts (CAFs) are pivotal in molding the cancer surroundings through their interactions with cancer cells. Recent research unveiled that cancer cells release a substance called SDF-1, which influences CAFs, fueling cancer cells' enhanced invasion, migration, and growth. Conversely, normal fibroblasts (NFs) impede these cancer cell activities. The process through which NFs evolve into CAFs and whether this transformation sparks cancer-promoting behavior remains uncertain. [4]. MicroRNAs (miRNAs) are brief non-coding RNAs consisting of 21–24 nucleotides. miRNAs regulate target genes by binding to mRNA 3'-UTRs, hence regulating post-transcriptional regulation of certain genes [5]. Research indicates that miRNA expression is disrupted in various cancer types due to over 50% of the genes governing miRNA production residing in fragile chromosomal regions, making them more prone to deletions or rearrangements. Misregulation of miRNA expression can impact cell differentiation and proliferation, allowing miRNAs to function as oncogenes or tumor suppressor genes. [6]. In recent studies, researchers have looked closely at miRNAs as potential signaling molecules or communication tools within the tumor microenvironment. [7]. MiRNAs can inhibit gene expression posttranscriptionally by binding to target mRNAs by base pairing with the 3'-untranslated region (UTR), leading to translational repression. Various mechanisms causing aberrant miRNA expression in cancer have been documented, including chromosomal rearrangements and epigenetic alterations [8]. Additional examinations, such as miRNA arrays, were employed to distinguish distinct miRNA patterns in exosomes obtained from normal fibroblasts (NFs) and cancer-associated fibroblasts (CAFs). We showed the direct transfer of miR-196a from cancer-associated fibroblasts (CAFs) to head and neck cancer (HNC) cells by exosomes. By looking at the molecular processes involved, we figured out how exosomal miR-196a influences the ability of head and neck cancer cells

to resist cisplatin [9]. Head and neck cancer, particularly squamous cell carcinoma, typically affects adults who are middle-aged or older, and it often has a grim outlook. Additionally, there has been a rise in the number of cases among individuals under the age of 45 [4]. Treatments for head and neck cancers include surgery, radiation therapy, and chemotherapy. However, these treatments can sometimes result in oral cavity issues, greatly affecting a patient's quality of life [4]. Oral squamous cell carcinoma (OSCC) is the most common malignant tumor in the head and neck region [1]. By understanding how oral squamous cell carcinoma (OSCC) forms and progresses, we might discover markers for diagnosis and find better targets for treating OSCC effectively. Prior research has primarily concentrated on the tumor cell, revealing various gene changes linked to OSCC formation and progression, including p53 inactivation [10]. Early identification methods and targeted treatment plans for oral squamous cell carcinoma (OSCC) are essential for reducing morbidity and mortality rates and enhancing patients' well-being. Carcinoma-associated fibroblasts (CAFs) exhibit continuous activity and significantly impact tumor development. Oral squamous cell carcinoma (OSCC) transforms human oral mucosal fibroblasts (hOMF) into cancer-associated fibroblasts (CAFs) by the release of platelet-derived growth factor-BB (PDGF-BB). The activated cancer-associated fibroblasts demonstrate significant tumorigenic characteristics [10]. Oral squamous cell carcinoma (OSCC) represents around 40% of head and neck malignancies and ranks as the sixth most often diagnosed cancer globally. Individuals with oral squamous cell carcinoma (OSCC) have poor clinical outcomes, and the 5-year survival rate for OSCC has remained stagnant for the last three decades [11]. Comprehending the molecular elements of OSCC is essential for creating innovative therapy approaches for OSCC. Prior research has demonstrated the importance of cancer cells and neighboring stromal cells in tumor progression [11]. This work aims to evaluate the impact of CAF-derived miRNAs on tumor microenvironments in head and neck malignancies through a comprehensive review.

Methods

This systematic review study was conducted following the PRISMA statement; the protocol of this study was registered on open science framework (OSF) DOI <https://doi.org/10.17605/OSF.IO/7E8K2>

Search strategy

We searched for original English-language articles published up to October 2023 in PubMed, Scopus, and WOS databases; Google Scholar was also checked for possible

relative data. Two reviewers (PM and PG) developed the search strategy as follows: ("Cancer-Associated Fibroblasts" OR "CAF" OR "CAFs") AND ("miRNA*" OR "microRNA*" OR "miR"). Table 1 shows the search strategies for PubMed, Scopus, Google Scholar, and WOS.

Inclusion criteria

In this study, the included and excluded items are as follows:

- Animal articles were excluded.
- Non-English language articles were excluded.
- Review articles were excluded.
- Original articles were included.
- Duplicate articles were excluded.

Data screening and extraction

This study was reviewed by title and abstract by two authors (PM and PG) in a blinded manner using the intelligent system for systematic reviews Rayyan. In cases where a disagreement between the two initial reviewers couldn't be resolved, a third reviewer (author MA) was brought in to evaluate the specific point of contention. This reviewer was independent of the initial review process, providing an objective perspective to help resolve the issue.

Two independent reviewers (PM and PG) screened the titles and abstracts of studies identified through our systematic search. Studies were included if they met the following criteria: (1) focused on head and neck malignancies, (2) investigated the role of cancer-associated fibroblast (CAF)-derived miRNAs, and (3) provided data on miRNA expression levels or related functional pathways. Full-text articles were retrieved for studies that met the initial inclusion criteria.

Data were independently extracted by the two reviewers (PM and PG) using a standardized data extraction form. For each included study, the following data were extracted:

Study characteristics: Author(s), year of publication, study design, sample size, and cancer type (e.g., oral squamous cell carcinoma, nasopharyngeal carcinoma).

MiRNA information: Specific miRNAs investigated, methods of miRNA isolation, miRNA expression analysis techniques (e.g., qRT-PCR, microarray, RNA sequencing), and the tissue types analyzed (e.g., tumor tissue, serum, exosomes).

Outcome measures: The reported role of the miRNA in the context of CAF-cancer interactions, including

its potential to regulate tumor progression, metastasis, or treatment resistance. Information on the clinical significance (e.g., survival rates, response to treatment) was also extracted if available.

Quality assessment: Quality scores based on the Scirap tool were recorded for each study, assessing the risk of bias and methodological rigor.

Quality assessment of included studies

The quality assessment of this study was done using the Scirap tool (<http://www.scirap.org>); the results are summarized in Table 2.

Results

Study selection

After searching the databases, 921 articles were identified. Following the removal of duplicates, 883 articles met the criteria for analysis. Articles were screened based on title/abstract. Eight hundred fifty-five publications were eliminated for irrelevance, and 28 articles meeting the inclusion criteria were approved for full-text analysis. Regrettably, the complete text of 7 papers was inaccessible to us, resulting in the inclusion of 21 articles in this evaluation. Refer to the PRISMA flow diagram for specifics on study identification and inclusion/exclusion (Fig. 1). A summary of the characteristics of the included articles is presented in Table 1.

Findings

The systematic review covered publications published between 2015 and 2023, all in English.

71.4% of the 21 articles in this survey were based on research carried out in China. Two articles, which account for 9.5% of the total, were authored by Iranian scholars. Four additional papers originated from Spain, Japan, Brazil, and Norway, collectively accounting for 19%. Ten articles were designated for in vitro studies, while eleven were assigned to in vitro-in vivo studies. OSCC and ESCC were the most prevalent forms of head and neck malignancies reviewed, with 9 and 5 papers, respectively. HNSCC and HNC were each mentioned in 2 articles. Three other publications analyzed different forms of squamous cell carcinoma of the skin, nasopharyngeal carcinoma, and esophageal cancer. The information is condensed in Table 1.

Outcome

Oral squamous cell carcinoma (OSCC)

Nine papers reviewed the role of different miRNAs in OSCC [1, 2, 6, 8, 10, 11, 14, 16, 23]. miR-21-5p exhibited the best statistical performance in differentiating tumor tissue and healthy mucosa [6]. MiR-21-5p was identified

Table 1 Search strategies and results of searching

Data base	Search strategy	results
pubmed	("Cancer-Associated Fibroblasts"[Title/Abstract] OR "CAF"[Title/Abstract] OR "CAFs"[Title/Abstract] OR "Cancer-Associated Fibroblasts"[MeSH Terms]) AND ("mirna*" [Title/Abstract] OR "microRNA*" [Title/Abstract] OR "MicroRNAs"[MeSH Terms]) AND ("head and neck malignanc*" [Title/Abstract] OR "head cancer" [Title/Abstract] OR "neck cancer" [Title/Abstract] OR "head and neck carcinoma" [Title/Abstract] OR "head carcinoma" [Title/Abstract] OR "neck carcinoma" [Title/Abstract] OR "laryngeal cancer" [Title/Abstract] OR "hypopharyngeal cancer" [Title/Abstract] OR "lip and oral cavity cancer" [Title/Abstract] OR "lip cancer" [Title/Abstract] OR "oral cancer" [Title/Abstract] OR "oropharyngeal cancer" [Title/Abstract] OR "salivary gland cancer" [Title/Abstract] OR "paranasal sinus and nasal cavity cancer" [Title/Abstract] OR "paranasal sinus cancer" [Title/Abstract] OR "nasal cavity cancer" [Title/Abstract] OR "Head and Neck Neoplasms"[MeSH Terms] OR "Laryngeal Neoplasms"[MeSH Terms] OR "Hypopharyngeal Neoplasms"[MeSH Terms] OR "Squamous Cell Carcinoma of Head and Neck"[MeSH Terms] OR "Lip Neoplasms"[MeSH Terms] OR "Oropharyngeal Neoplasms"[MeSH Terms] OR "Salivary Gland Neoplasms"[MeSH Terms] OR "Paranasal Sinus Neoplasms"[MeSH Terms] OR "Nasopharyngeal Neoplasms"[MeSH Terms]) OR "Nasopharyngeal Carcinoma"[MeSH Terms])	37
WOS	((TS= ("Cancer-Associated Fibroblast*") OR TS= (CAF) OR TS= (CAFs)) AND ((TS= (mirna*) OR TS= (microRNA*)) OR TS= ("head and neck malignanc*") OR TS= ("head cancer") OR TS= ("head and neck carcinoma") OR TS= ("neck carcinoma") OR TS= ("laryngeal cancer") OR TS= ("hypopharyngeal cancer") OR TS= ("lip and oral cavity cancer") OR TS= ("lip cancer") OR TS= ("oral cancer") OR TS= ("oropharyngeal cancer") OR TS= ("salivary gland cancer") OR TS= ("paranasal sinus and nasal cavity cancer") OR TS= ("paranasal sinus cancer") OR TS= ("nasal cavity cancer") OR TS= ("metastatic squamous neck cancer") OR TS= ("Squamous Cell Carcinoma of Head and Neck") OR TS= ("Head Neoplasms") OR TS= ("Neck Neoplasms") OR TS= ("Laryngeal Neoplasms") OR TS= ("Hypopharyngeal Neoplasms") OR TS= ("Mouth Neoplasms") OR TS= ("Lip Neoplasms") OR TS= ("Oropharyngeal Neoplasms") OR TS= ("Salivary Gland Neoplasms") OR TS= ("Paranasal Sinus Neoplasms") OR TS= ("Nasopharyngeal Neoplasms") OR TS= ("Nasopharyngeal Carcinoma"))	21
Scopus	(TITLE-ABS-KEY("Cancer-Associated Fibroblast*") OR TITLE-ABS-KEY(CAF) OR TITLE-ABS-KEY(CAFs)) AND (TITLE-ABS-KEY(mirna) OR TITLE-ABS-KEY(microRNA) OR TITLE-ABS-KEY(miRNA)) AND (TITLE-ABS-KEY("head and neck malignanc*") OR TITLE-ABS-KEY("head malignanc*") OR TITLE-ABS-KEY("head carcinoma") OR TITLE-ABS-KEY("neck cancer") OR TITLE-ABS-KEY("head and neck carcinoma") OR TITLE-ABS-KEY("laryngeal cancer") OR TITLE-ABS-KEY("hypopharyngeal cancer") OR TITLE-ABS-KEY("lip and oral cavity cancer") OR TITLE-ABS-KEY("lip cancer") OR TITLE-ABS-KEY("oral cancer") OR TITLE-ABS-KEY("oropharyngeal cancer") OR TITLE-ABS-KEY("salivary gland cancer") OR TITLE-ABS-KEY("paranasal sinus and nasal cavity cancer") OR TITLE-ABS-KEY("paranasal sinus cancer") OR TITLE-ABS-KEY("nasal cavity cancer") OR TITLE-ABS-KEY("metastatic squamous neck cancer") OR TITLE-ABS-KEY("Squamous Cell Carcinoma of Head and Neck") OR TITLE-ABS-KEY("Head and Neck Neoplasms") OR TITLE-ABS-KEY("Laryngeal Neoplasms") OR TITLE-ABS-KEY("Hypopharyngeal Neoplasms") OR TITLE-ABS-KEY("Mouth Neoplasms") OR TITLE-ABS-KEY("Lip Neoplasms") OR TITLE-ABS-KEY("Oropharyngeal Neoplasms") OR TITLE-ABS-KEY("Salivary Gland Neoplasms") OR TITLE-ABS-KEY("Paranasal Sinus Neoplasms") OR TITLE-ABS-KEY("Nasopharyngeal Neoplasms") OR TITLE-ABS-KEY("Nasopharyngeal Carcinoma"))	47
Google Scholar	Search strategy 1	allintitle: "head and neck cancer" mirna OR mirnas OR microrna OR micromas OR mir OR "Cancer Associated Fibroblast" OR "Cancer Associated Fibroblasts" OR caf OR cafs 816
	Search strategy 2	allintitle: mirna OR microrna OR mir OR "head and neck malignanc" OR "head malignanc" OR "oral cancer" OR "Salivary Gland Neoplasms" OR "paranasal sinus cancer" OR "head and neck cancer" "Cancer associated fibroblast" 816
	Search strategy 3	allintitle: CAF mirna OR microrna OR mir OR "head and neck malignanc" OR "head malignanc" OR "oral cancer" OR "Salivary Gland Neoplasms" OR "paranasal sinus cancer" OR "head and neck cancer" 816
	Search strategy 4	allintitle: "oral cancer" mirna OR mirnas OR microrna OR micromas OR mir OR "Cancer Associated Fibroblast" OR "Cancer Associated Fibroblasts" OR caf OR cafs 816
Total		921

Table 2 Summary characteristics of included studies

Author	Year	Country	Type of Study	Cancer type	Tissue sample	Tissue processing technique	Observed role of CAF	Conclusion
Alvarez-Teijeiro, et al. [12]	2017	Spain	In vitro (Human)	head and neck squamous cell carcinomas (HNSCC)	1. Case sample: Fresh frozen surgical tissue specimens from 19 patients with HNSCC. Control sample: Normal epithelia from non-oncologic patients not exposed to tobacco carcinogens (e.g., childhood tonsillectomy) Case sample: Formalin-fixed paraffin-embedded (FFPE) tissue samples from 40 patients diagnosed with laryngeal dysplasia. Control sample: FFPE normal epithelium 3. Case sample: Saliva samples were taken in advance from 15 patients with head and neck squamous cell carcinoma. Control sample: Saliva samples were collected from 11 healthy donors	1. Analysis of mRNA expression 2. miRNA target analysis using real-time RT-PCR 3. Cell Culture 4. Transfections using pre-miR miRNA precursors 5. Proteome Array and Western blot Analysis 6. Cell proliferation tests 7. Scratch-Induced Directional Migration Assay 8. Group three-dimensional spheroid invasion tests 9. Data Analysis	• miR-196a and miR-196b were shown to uniquely influence target genes and regulatory pathways in cells, affecting cell proliferation, migration, and invasion • The diverse impacts of miR-196a/b on distinct subcategories of HNSCC cells and neighboring CAFs might hinder the practicality of therapeutic interventions	• miR-196b dysregulation is an early event in HNSCC tumor formation, indicating its potential for early detection, disease monitoring, and use as a non-invasive biomarker in saliva • The diverse impacts of miR-196a/b on distinct subcategories of HNSCC cells and neighboring CAFs might hinder the practicality of therapeutic interventions
Khazaei et al. [7]	2017	Iran	In vitro (Human)	esophageal squamous cell carcinoma (ESCC)	Case sample: We gathered 26 sets of fresh-frozen tissues and 39 blood serum samples from patients diagnosed with esophageal squamous cell carcinoma (ESCC) at Sayad Shirazi Hospital, part of Golestan University of Medical Sciences in Iran The KYSE-30 cell line (human ESCC) and HFSF-P13 cell line (human normal fibroblasts from skin) were obtained from the National Cell Bank of Iran (Pasteur Institute)	1. Isolation of RNA from esophageal tissue, serum samples, and purified exosomes Quantification of miR-451 using quantitative reverse transcription polymerase chain reaction (RT-PCR) 3. Exosome isolation 4. Cell culture conditions and miR-451 overexpression 5. Co-cultivation of an esophageal cancer cell line with normal fibroblasts (HFSF-P13) 6. Migration Analysis Analyzing MIF expression in the co-cultured cell lines 8. Statistical analyses	• Serum samples from patients with esophageal cancer exhibited elevated levels of miR-451. Subsequent analysis of cryopreserved tumor tissues from the same patients revealed reduced miR-451 expression • When the KYSE-30 cell line was co-cultured with normal fibroblasts, there was a notable increase in the release of miR-451 exosomes into the culture medium. When the KYSE-30 cell line was co-cultured with fibroblasts expressing high amounts of miR-451, there was a significant increase in the migratory capacity of the KYSE-30 cells. Line • The MIF expression in the KYSE-30 cell line showed an increase, although it was not statistically significant. MIF is a confirmed target of miR-451	• The study shows that cancer-associated fibroblasts use exosomal miR-451 as a signaling molecule to promote tumor cell migration and accelerate cancer progression

Table 2 (continued)

Author	Year	Country	Type of Study	Cancer type	Tissue sample	Tissue processing technique	Observed role of CAF	Conclusion
Chunping Wu1 – Et al [13]	2022	China	In vitro (Human)	supraglottic laryngeal squamous cell carcinoma (SLSCC)	Case sample: Ten tumor specimens from 10 male patients with SLSCC	1. Primary culture of tumor specimens and corresponding surrounding connective tissues 2. Morphological analysis and immunocytochemical labeling of cancer-associated fibroblasts (CAFs) and normal fibroblasts (NFs) 3. Harvesting culture supernatant and isolating exosomes 4. Utilizing transmission electron microscopy (TEM) and nanoparticle tracking analysis (NTA) 5. Immunoblotting 6. Total RNA extraction 7. Analysis of small RNA (sRNA) sequencing and differential expression 8. Real-time quantitative polymerase chain reaction (RT-qPCR) confirmation of specific miRNAs 9. Identification of potential exosomal miRNAs and their target genes 10. Analysis of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) 11. Statistical analysis	• Exosomal miRNAs secreted by cancer-associated fibroblasts (CAFs) exhibited reduced expression levels, such as miR-656-3p, miR-337-5p, miR-29a-3p, and miR-655-3p. Conversely, miR-184-3p, miR-92a-1-5p, miR-212-3p, and miR-3135b exhibited elevated expression levels • The KEGG analysis identified 30 crucial pathways associated with cancer start, progression, and cell cycle regulation • The interaction network identified miR-16-5p, miR-29a-3p, miR-34c-5p, miR-32-5p, and miR-490-5p as the top five miRNAs, and CCND1, CDKN1B, CDK6, PTEN, and FOS as the top five target genes	• SLSCC patients exhibited unusual expression of miRNAs originating from exosomes of cancer-associated fibroblasts • Identifying the top five miRNAs and their target genes may suggest an unfavorable tumor environment and act as therapy indicators for SLSCC

Table 2 (continued)

Author	Year	Country	Type of Study	Cancer type	Tissue sample	Tissue processing technique	Observed role of CAF	Conclusion
Shen et al. [4]	2017	China	In vitro (Human)	Head and neck cancer (HNC)	Case example: 1. Cancer-associated fibroblasts (CAFs) and normal fibroblasts (NFs) were obtained from patients who had surgery for head and neck cancers at the Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine The Dental School of the University of Maryland generously supplied HN13 cells	1. Establishing primary cell cultures of cancer-associated fibroblasts (CAFs) and normal fibroblasts (NFs) 2. Culturing cells from head and neck cancer 3. Immunoblotting 4. Immunofluorescence and immunohistochemistry 5. Cell cycle assessment 6. Analysis of miRNA array 7. Real-time PCR test 8. Migration test 9. Co-culturing of Head and Neck Cancer (HNC) cells with Cancer-Associated Fibroblasts (CAFs) or Normal Fibroblasts (NFs) Construction of the RASSF2 luciferase reporter gene vectors 11. Data analysis	• miR-7 expression was shown to be increased in cancer-associated fibroblasts (CAFs) • Increased miR-7 levels reduced RASSF2 gene expression, reducing PAR-4 release in the cancer microenvironment • Cancer-associated fibroblasts (CAFs) reducing the release of PAR-4 may lead to enhanced proliferation and migration of cancer cells in the tumor microenvironment	• Targeting the disruption of the RASSF2-PAR-4 axis by miR-7 might be an innovative approach for gene therapy in head and neck cancers, as shown by the results

Table 2 (continued)

Author	Year	Country	Type of Study	Cancer type	Tissue sample	Tissue processing technique	Observed role of CAF	Conclusion
Min et al. [14]	2016	China	In vitro (human)	oral squamous cell carcinoma (OSCC)	Example case: 1. Nucleated fibroblasts (NFs) and cancer-associated fibroblasts (CAFs) were extracted from oral squamous cell carcinoma (OSCC) tumor tissues and identified based on specific markers 2. The human oral cancer SCC-25 cells were acquired from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences	1. Cell Line and Culture Transfection 2. Plasmids and Cellular Transfection 3. Real-Time PCR Analysis 4. Western Blot Analysis 5. Conduct Luciferase Reporter Assay 6. WNT10B Transfection and Immunodepletion 7. Cell Invasion and Migration Assay 8. Data Analysis	• Expression of miR-7 was shown to be elevated in cancer-associated fibroblasts (CAFs) • Elevated miR-7 levels suppressed the expression of the target gene RASSF2, resulting in decreased PAR-4 release in the cancer microenvironment • Cancer-associated fibroblasts (CAFs) decreasing the secretion of PAR-4 might result in increased cancer cell proliferation and movement within the tumor microenvironment • miR-148a expression was decreased in cancer-associated fibroblasts (CAFs) compared to normal fibroblasts obtained from clinical oral squamous cell carcinoma (OSCC) tissue • Increasing miR-148a levels in cancer-associated fibroblasts (CAFs) considerably hindered the movement and penetration of oral carcinoma cells (SCC-25) by focusing on WNT10B	The findings suggest that miR-148a may be a promising target for treating OSCC
Nouraei et al. [15]	2016	Iran	In vitro (human)	esophageal squamous cell carcinoma (ESCC)	Case sample: The esophageal squamous cell carcinoma cell line, KYSE-30, and the human regular fibroblasts cell line, HFSF-P13, were acquired from the National Cell Bank of Iran at the Pasteur Institute in Tehran	1. Cell lines and co-culture system 2. Preparation of conditioned medium (CM) 3. Isolation of RNA and synthesis of cDNA 4. Amplification and profiling of MicroRNA 5. Analysis of data processing and pathway enrichment Purification of exosomes via ultracentrifugation Perform quantitative real-time polymerase chain reaction (qRT-PCR) on exosomal materials	• Discovered 18 miRNAs that exhibited substantial over-expression or downregulation in the conditioned medium of the co-culture system • Pathways related to cell adhesion, endocytosis, and cell junctions may be connected to the CAF phenotype and tumor progression • High amounts of miR-33a and miR-326 were found in the exosomes recovered from a conditioned medium of co-cultured and untreated cells	Their research clarified the role of cancer-associated fibroblasts (CAFs) in the tumor microenvironment through the secretion of miRNAs, proposing novel therapy options for esophageal and other types of cancer

Table 2 (continued)

Author	Year	Country	Type of Study	Cancer type	Tissue sample	Tissue processing technique	Observed role of CAF	Conclusion
LHPING SUN- et al. [1]	2019	China	In vitro (Human)	Oral squamous cell carcinoma (OSCC)	Case example: 1. Participants and tissue specimens Forty-seven patients diagnosed with oral squamous cell carcinoma (OSCC), including 27 men and 20 women aged 39 to 72 years, who underwent tumor removal surgery at Liaocheng People's Hospital's Department of Oral Maxillofacial Surgery from January 1, 2014, to December 31, 2017 2. Culturing cells Oral squamous cell carcinoma The CAL-27 cells were acquired from the American Type Culture Collection (ATCC; Manassas, VA, USA)	1. Culturing cells 2. Fibroblast isolation 3. OSCC cells co-cultured with CAFs or NFs 4. Immunohistochemistry (IHC) 5. Immunocytochemistry 6. Isolation of exosomes 7. Conducting Western blot analysis 8. Transfection 9. Isolation of RNA and quantitative real-time PCR 10. Conducting Transwell migration and invasion tests 11. Analysis using flow cytometry Identifying potential targets of miR-382-5p in Targetscan 13. Data analysis	- miR-382-5p levels were higher in cancer-associated fibroblasts (CAFs) than in fibroblasts from nearby healthy tissue. This increase in miR-382-5p was associated with the migration and invasion of oral squamous cell carcinoma (OSCC) cells - It was shown that exosomes from cancer-associated fibroblasts carried miR-382-5p to oral squamous cell carcinoma cells	Exosomes derived from cancer-associated fibroblasts transfer miR-382-5p to oral squamous cell carcinoma cells, enhancing their ability to move and invade. The work validated a novel mechanism via which cancer-associated fibroblasts (CAFs) promote oral squamous cell carcinoma (OSCC) proliferation, providing possible avenues for cancer therapy
Junior et al. [6]	2021	Brazil	In vitro (human)	squamous cell carcinoma of the oral cavity (OSCC)	Case sample: We selected 26 cases and collected remaining FFPE blocks containing oral cavity squamous cell carcinoma (SCC) samples from patients who underwent primary curative resection at the Head and Neck Surgery Service of the Cancer Institute of the State of São Paulo (ICESP) in São Paulo, Brazil, between December 2010 and October 2015 Control sample: Tissues from the oral mucosa preserved in FFPE blocks were taken from 11 individuals who underwent operations for benign oral cavity disorders, forming the control group	1. Technique for detecting MicroRNA 2. Immunohistochemical analysis 3. Data analysis	- Elevated levels of miR-21-5p and miR-106-5p, together with decreased levels of miR-320a and miR-222-3p, suggested malignancy - When examined separately, miR-21-5p had the best statistical performance in differentiating between tumor tissue and healthy mucosa	This work demonstrates the influence of microRNAs on the progression of OSCC and the notable alterations they induce in the tumor microenvironment

Table 2 (continued)

Author	Year	Country	Type of Study	Cancer type	Tissue sample	Tissue processing technique	Observed role of CAF	Conclusion
Saroj et al. [16]	2021	Norway	In vitro (human)	oral squamous cell carcinoma (OSCC)	Case study: Before any treatment, CAFs were extracted from biopsies of OSCC primary lesions from 13 patients at Haukeland University Hospital in Bergen, Norway acquired matched normal oral fibroblasts (NOFs) from biopsies of cancer-free areas of the oral mucosa from five OSCC patients Two OSCC cell lines, UK1 and Luc4, were used Control samples were taken from biopsies of healthy volunteers' normal oral mucosa without cancer (n = 9)	1. Fibroblast isolation and cell culture 2. Extraction of total RNA and enrichment of small RNA for miR microarray analysis 3. miR Microarray 4. Transcription Reversal 5. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) 6. Analysis of TCGA Data 7. Identification of miRNA Targets and Dual Luciferase Target Reporter Assay for miRNAs 8. Modulation of miR-204 in Cultured Fibroblasts 9. Isolation and Quantification of Proteins 10. Immunoblotting 11. 2D Co-Culture Migration Assay 12. Collagen Contraction Assay 13. 3D Organotypic Co-Cultures Quantifying the invasion of oral squamous cell carcinoma (OSCC) cells in 3D-organotypic models 15. Data Analysis	Modifying miR-204 expression did not affect fibroblast cell proliferation but did induce changes in cell motility properties; the concentrations of several molecules linked to cell motility, and the invasion of adjacent OSCC cells. Experimental validation using a 3' UTR miR target reporter assay demonstrated that ITGA11 is directly targeted by miR-204	The study identified certain miRs with varying expression levels in stromal fibroblasts of OSCC lesions in comparison to normal oral mucosa. One of the miRs significantly decreased in cancer-associated fibroblasts (CAFs), miR-204, hinders the movement of fibroblasts and contributes to the suppression of malignancies. This is achieved by regulating the synthesis of several molecules and specifically targeting ITGA11
Matos et al. [17]	2020	China	In vitro (human)	Oral squamous cell carcinoma (OSCC)	Case example: 26 patients were chosen and categorized into two groups: 1. The superficial tumor group consisted of 12 neoplasms with a DOI of 5 mm. 2. The deep tumor group comprised 14 neoplastic patients with a DOI of 15 and 25 mm Control sample: Eleven individuals with similar epidemiological characteristics gave healthy oral mucosa samples collected from oral surgery for benign mouth diseases, such as mucocoeles or fibromas of the mucosa	1. MicroRNA detection technique 2. Immunohistochemical analysis 3. Statistical analysis	- miR-1-3p, miR-133-3p, and miR-21-5p showed varying expression levels in the superficial and deep tumor categories - miR-21-5p had the best accuracy in distinguishing between superficial and deep cancers. This method was linked to decreased miR-1-3p levels and increased miR-21-5p levels - Patients with elevated levels of miR-133a-3p had improved overall and disease-free survival rates - The study demonstrated the correlation between microRNAs and the restructuring of the extracellular matrix in oral squamous cell cancer	- Deep OSCC specimens exhibited decreased levels of miR-1-3p and miR-133a-3p, together with increased levels of miR-21-5p, indicating potential involvement of these miRNAs in OSCC tumor invasion compared to superficial tumors - Patients with elevated miR-133a-3p levels had better overall and disease-free survival rates, whereas miR-1-3p expression was linked to greater disease-free survival

Table 2 (continued)

Author	Year	Country	Type of Study	Cancer type	Tissue sample	Tissue processing technique	Observed role of CAF	Conclusion
Chen et al. [5]	2023	China	In vitro (Human) and In vivo (animal)	esophageal squamous cell carcinoma (escc)	Ex vivo Case sample: ESCC tissues surgically excised were gathered for analysis. Six fresh and 53 paraffin-embedded samples of ESCC tissue without necrosis were chosen Control sample: six fresh and 25 paraffin-embedded normal esophagus tissue samples Twenty-five female BALB/c nude mice were acquired from Beijing Vital River Laboratory Animal Technology Co., Ltd. for in vivo experiments	1. Hematoxylin-eosin (HE) staining and immunohistochemistry 2. Cell culture 3. Fibroblast extraction 4. Western blotting 5. Immunofluorescence staining 6. Exosome purification 7. Transmission electron microscopy 8. Analysis of particles using NanoSight particle tracking 9. Cultivation of Human Lens Epithelial Cells in a Prepared Growth Environment 10. Labeling and tracking exosomes 11. Cell migration analysis 12. Cell invasion assay 13. Cell proliferation analysis 14. Cell tube formation assay 15. RNA isolation and quantitative real-time polymerase chain reaction (qRT-PCR) 16. Gene transfection Dual-luciferase reporter assay 18. Fluorescence in situ hybridization 19. Mouse models of tumors and their treatment 20. Statistical analysis	• Cancer-associated fibroblast-derived exosomes promoted lymphatic endothelial cells' proliferation, migration, invasion, and tube formation. Additionally, they stimulated lymphangiogenesis in ESCC xenografts • Levels of miR-100-5p were much lower in exosomes from cancer-associated fibroblasts than in exosomes from normal fibroblasts • miR-100-5p inhibited the proliferation, migration, invasion, and angiogenic potential of TLECs. Additionally, miR-100-5p inhibited the development of lymphatic vessels in ESCC xenografts	Exosomes derived from cancer-associated fibroblasts with decreased miR-100-5p levels can enhance lymphatic channel development, suggesting a possible strategy to inhibit lymphatic dissemination in esophageal squamous cell carcinoma by focusing on the IGF1R/PI3K/AKT pathway

Table 2 (continued)

Author	Year	Country	Type of Study	Cancer type	Tissue sample	Tissue processing technique	Observed role of CAF	Conclusion
Koji Tanaka – et al. [18]	2015	Japan	In vivo (human) and In vitro (human)	esophageal squamous cell carcinoma (escc)	An in vivo case The study involved 64 patients who received neoadjuvant chemotherapy and underwent surgery for primary esophageal squamous cell carcinoma at the Department of Gastroenterological Surgery, Graduate School of Medicine, Osaka University In vivo control sample: 27 healthy participants Sample case in vitro: The verified human esophageal squamous cell line TE10 was acquired from the Riken Bioresource Center Cell Bank in Tsukuba, Japan	1. Assessment of the reaction to chemotherapy 2. Isolation and characterization of fibroblasts 3. Cell lines and culture conditions 4. RNA isolation 5. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) for measuring miRNA levels 6. Quantitative real-time polymerase chain reaction 7. miRNA transfection 8. microRNA array 9. Immunofluorescence staining 10. Conducting growth inhibition experiments using cisplatin therapy and apoptosis assay 11. ELISA 12. Western blotting 13. Statistical analysis	- Increased levels of miR-27a/b expression correlated with a poor response to chemotherapy in patients with esophageal cancer - Introducing miR-27a/b into cancer cells did not substantially impact their reaction to chemotherapy - Esophageal cancer cells cultured in the medium surrounding normal fibroblasts transfected with miR-27a/b exhibited reduced responsiveness to cisplatin compared to cells cultured in the medium surrounding normal fibroblasts - Transfected normal fibroblasts with MiR-27a/b showed elevated α-smooth muscle actin (α-SMA) expression, a characteristic of cancer-associated fibroblasts (CAF), and increased levels of transforming growth factor-β (TGF-β)	miR-27a/b contributes to chemotherapy resistance in esophageal cancer by inducing the conversion of normal fibroblasts into cancer-associated fibroblasts (CAF)

Table 2 (continued)

Author	Year	Country	Type of Study	Cancer type	Tissue sample	Tissue processing technique	Observed role of CAF	Conclusion
Qitai Zhao – et al. [3]	2021	China	In vitro (human) and In vivo (Human)	esophageal squamous cell carcinoma (ESCC)	Case sample: Tumor specimens and peripheral blood were collected from patients with esophageal squamous cell carcinoma (ESCC) at the First Affiliated Hospital of Zhengzhou University. Peripheral blood was collected from individuals with esophageal squamous cell carcinoma who had four rounds of cisplatin-based treatment. The regular skin fibroblast cell line (HSC-1) and tumor cell lines (EC1 and KYSE70) were acquired from the Chinese Academy of Sciences Cell Repository in Shanghai, China	1. In vitro cell cultivation 2. Isolation and cultivation of primary cancer-associated fibroblasts. 3. Co-culture study 4. Conduct Western blot analysis 5. Flow cytometry analysis 6. Isolation and purification of exosomes 7. Exosome identification 8. Analysis of Exosome Uptake 9. Analysis using immunohistochemistry 10. Immunofluorescence staining 11. Real-time PCR Transfection assay using miR-21 mimics and inhibitors 13. Analysis of survival 14. Analysis with Cytometric Bead Array 15. Analysis of bioinformatics 16. Statistical analysis	- Research has shown that IL-6 and exo-miR-21, produced by cancer-associated fibroblasts, converted monocytes into monocytic-myeloid-derived suppressor cells by collectively activating the STAT3 signaling pathway - Elevated levels of M-MDSCs and CAFs in tumor tissue correlated with resistance to DDP and a reduced estimated overall survival in patients with ESCC	- Cancer-associated fibroblasts release exosomes containing miR-21 which are transferred to monocytes. Exo-miR-21 is released into the cytoplasm and targets PTEN - IL-6 is acknowledged as the primary cytokine responsible for activating STAT3. MIR-21 stimulates STAT3 by targeting PTEN in coordination with IL-6. IL-6 and exo-miR-21 were discovered to enhance STAT3 phosphorylation, leading to the conversion of monocytes into M-MDSCs - Cancer-associated fibroblasts produced myeloid-derived suppressor cells that shielded tumor cells from cisplatin-induced cell death, resulting in unfavorable outcomes for patients with esophageal squamous cell carcinoma This highlights the crucial partnership between cancer-associated fibroblasts and myeloid-derived suppressor cells in enhancing medication resistance

Table 2 (continued)

Author	Year	Country	Type of Study	Cancer type	Tissue sample	Tissue processing technique	Observed role of CAF	Conclusion
Guquan Zhu – Et al [19]	2021	china	In vitro (human) and In vivo (animal)	head and neck squamous carcinoma (HNSCC)	Two human HNSCC cell lines, SCC-9 and CAL-27, were acquired from and verified by ATCC for in vitro experimentation MRC-5 cells were acquired from the China Center for Type Culture Collection Female Nude mice were acquired from the Laboratory Animal Center of Sichuan University in Chengdu, Sichuan, China, for the in vivo case sample	1. Cell culture under hypoxic conditions 2. Isolation of small extracellular vesicles (sEVs) and extraction of RNA 3. Scanning electron microscopy 4. Construction and sequencing of mRNA library (qRT-PCR) 5. Quantitative real-time PCR (qRT-PCR) 6. Immunofluorescence (IF) and immunohistochemistry (IHC) 7. Immunoblotting 8. Invasion analysis 9. Extracellular acidification rate (ECAR) 10. Utilization of cytokine array and Enzyme-Linked Immunosorbent Assay (ELISA) 11. Xenograft 12. ChIP assay 13. Data analysis	• Hypoxic head and neck squamous cell carcinoma cells induced the conversion of cancer-associated fibroblasts by releasing transforming growth factor beta (TGF-β) and small extracellular vesicles (sEVs) harboring elevated levels of miR-192/215 family microRNAs • Caveolin-1 (CAV1), a gene controlled by miR-192/215, inhibited the TGF-β/SMAD signaling pathway and promoted the transformation of fibroblasts into cancer-associated fibroblasts (CAFs). High levels of miR-192/215 in small extracellular vesicles (sEVs) from Head and Neck Squamous Cell Carcinoma (HNSCC) tissue, as opposed to blood serum, indicate the existence of hypoxic and aggressive malignant tissue • miR-215 was found in small extracellular vesicles (sEVs) from tumor tissue but not in circulating sEVs. It was linked to a worse overall survival rate in patients with head and neck squamous cell carcinoma (HNSCC) - Small extracellular vesicles (sEVs) carry miRNAs from cancer cells to fibroblasts, aiding in remodeling the hypoxic tumor microenvironment • Extracellular vesicles that are soluble and derived from cancerous tissue may serve as a potential source of biomarkers.	Hypoxia increases the concentrations of miR-192/215 in the small extracellular vesicles (sEVs). Fibroblasts absorb small extracellular vesicles (sEVs) with elevated miR-192/215 levels, resulting in reduced CAV1 expression and activation of TGF-β signaling. The regulatory mechanism enables cancer cells to induce the conversion of stromal fibroblasts into cancer-associated fibroblasts, promoting the progression of tumor cells in a feedback loop. Small extracellular vesicles (sEVs) deliver microRNAs to cancer cells and stromal fibroblasts, aiding in the remodeling of the hypoxic tumor microenvironment in the Head and Neck Squamous Cell Carcinoma (HNSCC). Solid extracellular vesicles (sEVs) derived from cancer tissues, rather than peripheral blood, have the potential to serve as vehicles for biomarkers

Table 2 (continued)

Author	Year	Country	Type of Study	Cancer type	Tissue sample	Tissue processing technique	Observed role of CAF	Conclusion
Li et al. [11]	2017	China	In vitro (human) and In vivo (animal)	Oral squamous cell carcinoma (OSCC)	In vitro case sample: 1. SCC-9 and CAL-27 cells were purchased from ATCC (American Type Culture Collection, Manassas, VA, USA) 2. Patient samples and cell isolation A total of 6 paired fresh, surgically resected OSCC tumors and matched non-malignant adjacent tissues were obtained from Chenzhou No.1 people's hospital In vivo case sample: Female BALB/c nude mice (4-week-old)	1. Cell Culture and Reagents isolation 2. Patient samples and cell isolation 3. Transfection of cells with pre-miRNA precursors or anti-miRNA inhibitors 4. Lentivirus transduction and establishment of stable cell lines 5. Quantitative real-time PCR 6. DNA methylation analysis 7. Luciferase reporter assay 8. Cell migration assay 9. CCL2 and IL-8 ELISA assay 10. Mouse xenograft models 11. Statistical analysis	- DNA hypermethylation leads to the downregulation of miR-124 in cancer-associated fibroblasts (CAFs), oral cavity carcinomas (OCCs), and oral squamous cell carcinoma (OSCC) tissues. Moreover, miR-124 plays a vital role in controlling the communication between cancer-associated fibroblasts (CAFs) and normal fibroblasts (NFs) and ovarian cancer cells (OCCs) via influencing the production of CCL2/IL-8 - Injecting miR-124 intravenously decreased oral tumor development in mice, indicating that increasing miR-124 levels might be a novel therapeutic strategy for treating oral cancer patients	miR-124 rescue could be a potential rationale for therapeutic applications in oral cancer

Table 2 (continued)

Author	Year	Country	Type of Study	Cancer type	Tissue sample	Tissue processing technique	Observed role of CAF	Conclusion
Li et al. [8]	2018	China	In vitro (human) and In vivo (animal)	oral squamous cell carcinoma (OSCC)	In vitro case sample: 1. Primary human CAFs and donor-matched NFs were isolated from OSCC patients treated by surgical resection at the Department of Oral and Maxillofacial Surgery, Guanghua School of Stomatology, Hospital of Stomatology, Sun Yat-sen University 2. Human oral keratinocytes (HOK) were purchased from ScienCell 3. OSCC cells, including CAL27 and SCC15, were kindly donated by the Department of Central Laboratory, Peking University, School and Hospital of Stomatology In vivo case sample: BALB/c nude mice (Beijing Vital River, Beijing, China) at 4–6 weeks of age were used	1. Isolation of primary human fibroblasts and OSCC cell culture 2. Exosome preparation 3. Preparation of lentiviral vector and transfection 4. Immunofluorescence 5. Quantitative Real-Time PCR 6. miRNA sequencing 7. Western blotting 8. Gelatin zymography 9. Functional assay 10. Luciferase reporter assay 11. In situ hybridization 12. Animal models 13. Statistical analysis	• The research showed a significant reduction in miR-34a-5p in exosomes from cancer-associated fibroblasts (CAFs) and proved that fibroblasts may transfer exosomal miR-34a-5p to oral squamous cell carcinoma (OSCC) cells • In xenograft studies, it was shown that increasing the levels of miR-34a-5p in cancer-associated fibroblasts (CAFs) inhibited the development of tumors from oral squamous cell carcinoma (OSCC) cells • miR-34a-5p was shown to hinder the proliferation and dissemination of OSCC cells by attaching to its specific target, AXL • Continuous abnormal synthesis of AXL in OSCC cells with increased levels of miR-34a-5p counteracted the decrease in cell growth and movement induced by the miRNA. The miR-34a-5p/AXL axis promoted the advancement of OSCC by activating the AKT/GSK-3β/catenin signaling pathway, resulting in the initiation of epithelial-mesenchymal transition (EMT) and boosting cancer cell metastasis. The miR-34a-5p/AXL pathway facilitated the translocation of β-catenin to the nucleus, resulting in enhanced SNAIL production through transcriptional upregulation. As a result, both MMP-2 and MMP-9 were activated	The miR-34a-5p/AXL axis confers aggressiveness in oral cancer cells through the AKT/GSK-3β/catenin/Snail signaling cascade and might represent a therapeutic target for OSCC

Table 2 (continued)

Author	Year	Country	Type of Study	Cancer type	Tissue sample	Tissue processing technique	Observed role of CAF	Conclusion
Qin, et al. [20,]	2019	china	In vitro (human) and In vivo (animal)	head and neck cancer (HNC)	In vitro case sample: 1. 80 pairs of tumor and adjacent normal tissues were obtained from patients diagnosed with primary HNC and underwent initial surgery between September 2011 and June 2015; another 28 pairs were collected between July 2018 and September 2018 2. In another 40 HNC patients, plasma samples were collected one day before surgery and three days after tumor resection 3. SCC-4, SCC-9, SCC-25, CAL 27, and 293 T cells were purchased from the American Type Culture Collection (ATCC, USA), and the University of Maryland Dental School, USA kindly provided the human HNC cell lines HN4, HN6, and HN30 In vitro control sample: Plasma samples from 30 donors who had undergone physical examination at the Ninth Peoples Hospital were selected as healthy controls In vivo case sample: A tumor-bearing model was constructed in BALB/C athymic nude mice (4 weeks old)	1. Cell cultures 2. Immunofluorescence 3. Western blot analysis 4. MTT assay 5. CM preparation 6. Exosome isolation 7. Transmission electron microscopy 8. Fluorescent labeling and transfer of exosomes 9. Co-culture assay 10. Plasmid construction 11. Cell transfection 12. Biotin miRNA pull-down assay 13. RNA extraction and real-time PCR analysis 14. RIP assay 15. Luciferase analysis 16. Immunoprecipitation of miRNA targets 17. Tumorigenicity assay in vivo 18. Statistical analyses	• Cancer-associated fibroblasts (CAFs) are naturally resistant to cisplatin and play a crucial role in controlling the survival and growth of head and neck cancer cells by transferring active miR-196a from CAFs to tumor cells through exosomes • Exosomal miR-196a interacts with new targets, CDKN1B and ING5, to provide resistance to cisplatin in head and neck cancer cells. Eliminating exosome or exosomal miR-196a from cancer-associated fibroblasts (CAFs) restored head and neck cancer (HNC) sensitivity to cisplatin • Cancer-associated fibroblast-derived exosomes may employ heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) to assist in packaging miR-196a • Higher levels of plasma exosomal miR-196a are associated with reduced overall survival rates and resistance to therapy	The study discovered that miR-196a from CAF-derived exosomes causes resistance to cisplatin in head and neck cancer via affecting CDKN1B and ING5. This suggests that miR-196a might be a valuable indicator and a possible target for overcoming cisplatin resistance in head and neck cancer

Table 2 (continued)

Author	Year	Country	Type of Study	Cancer type	Tissue sample	Tissue processing technique	Observed role of CAF	Conclusion
Jin Yang ¹ – Et al [21]	2021	china	In vitro (human) and In vivo (animal)	oral squamous cell carcinoma (OSCC)	In vitro case sample: 1. 6 OSCC patients (were obtained from the West China Hospital of Stomatology at Sichuan University during 2017–2019; The OSCC patients were 45–63 years old, experienced no relapses, and underwent no preoperative chemotherapy and/or radiotherapy) 2. The human OSCC cell lines Cal-27, UMSCC-1, HSC-2, and FaDu were purchased from ATCC or obtained from the State Key Laboratory of Oral Diseases In vitro control sample: 1. 6 patients who received third molar extraction as normal controls 2. Cal-27 cells were used alone as the control group in this study In vivo case sample: Four- to six-week-old BALB/c nude mice, half male and half female, were purchased from Charles River (Beijing, China)	1. Clinical tissue sample collection and primary cell culture 2. Plasmid construction and cell transfection 3. Cell lines and mice 4. RNA isolation, RNA sequencing, and qRT-PCR 5. Immunohistochemistry and immunofluorescence 6. Histology and immunohistochemistry 7. Western blotting 8. RNA-FISH and luciferase reporter assays 9. Bioinformatic analysis 10. Statistical analysis	Using siRNA to knock down lncRNA H19 suppressed the MAPK signaling pathway and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) and miR-675-5p. Additionally, the lncRNA H19/miR-675-5p/PFKFB3 axis was found to promote the glycolysis pathway in oral cancer-associated fibroblasts (CAFs), confirmed through luciferase reporter system assays and treatment with a specific miRNA inhibitor miR-675-5p/PFKFB3 act as key regulators in lncRNA H19-mediated glycolysis in oral CAFs	The lncRNA H19 was found to be a crucial lncRNA in oral cancer-associated fibroblasts (CAFs) and was simultaneously increased in both oral cancer cell lines and CAFs. Using small interfering RNA (siRNA) techniques, we found that reducing lncRNA H19 levels impacted the growth, movement, and glucose metabolism of oral cancer-associated fibroblasts (CAFs). The study introduces a novel approach to analyzing glucose metabolism in oral cancer-associated fibroblasts (CAFs), potentially offering a unique biomarker for oral squamous cell carcinoma (OSCC) diagnosis and a fresh target for anti-tumor treatment

Table 2 (continued)

Author	Year	Country	Type of Study	Cancer type	Tissue sample	Tissue processing technique	Observed role of CAF	Conclusion
Jin et al. [21]	2021	China	In vitro (human) and In vivo (animal)	Esophageal squamous cell carcinoma (ESCC)	In vitro case sample: 1. Samples of tumor tissue and precancerous tissue were collected from five patients who did not receive any preoperative chemotherapy or radiotherapy enrolled in the Cancer Institute and Hospital, Guangzhou Medical University (Guangzhou, China) 2. Patient-derived ESCC cell lines, human ESCC cell lines (EC-18 and KYSE30), and embryonic kidney cell line 293 (HEK293) were provided by Professor Guan from the Department of Clinical Oncology, The University of Hong Kong In vivo case sample: Four- to six-week-old female Balb/c mice were used to examine allograft tumor growth	1. Cell Culture 2. Isolation and Quantification of Exosomes 3. Cell migration, colony formation, and wound healing assays 4. Fluorescent immunostaining 5. MicroRNA sequencing 6. Quantitative real-time PCR 7. Western blot analysis 8. Animal experiments and welfare 9. Statistical analysis	• The mRNA level of hsa-miR-3656 showed a significant rise in these exosomes • Comparisons between tumor cell behavior in lab dishes and animals showed that the presence of miR-3656 significantly improved the proliferation, migration, and invasion capacities of esophageal squamous cell carcinoma (ESCC) cells • ACAP2 was discovered as a target gene controlled by miR-3656, detrimental to tumor development. Exosome-delivered miR-3656 suppresses ACAP2, leading to activation of the PI3K/AKT and β-catenin signaling pathways, promoting ESCC cell proliferation in laboratory settings and xenograft models	These findings indicate that exosomes carrying miR-3656 from cancer-associated fibroblasts (CAFs) play a role in the aggressive advancement of esophageal squamous cell carcinoma (ESCC). These results suggest a promising therapeutic target for ESCC and offer a fresh perspective for clinical treatment strategies

Table 2 (continued)

Author	Year	Country	Type of Study	Cancer type	Tissue sample	Tissue processing technique	Observed role of CAF	Conclusion
Li, et al. [22]	2022	China	In vitro (human) and In vivo (animal)	Nasopharyngeal carcinoma (NPC)	In vitro case sample: 1. Tumor tissues from thirty patients diagnosed with NPC by biopsy from January 2016 to June 2017 were collected at the Hainan Affiliated Hospital of Hainan Medical University 2. Human NPC cell lines CNE2, HNE1, HNE2, HONE1, C666-1, SUNE-1, nasopharyngeal epithelial NP69 cells, and 293 T cells provided by the National Collection of Authenticated Cell Cultures In vitro control sample: Thirty normal nasopharyngeal tissues In vivo case sample: Eight- to ten-week-old male BALB/c nude mice	1. Cell culture and treatment 2. Cell transfection 3. Conditioned medium (CM) preparation 4. Exosome isolation and characterization 5. Iodixanol density gradient centrifugation 6. Real-time quantitative reverse-transcription PCR (RT-qPCR) 7. Western blot 8. Cell Counting Kit-8 (CCK-8) assay 9. Colony formation 10. Wound healing assay 11. Transwell assay 12. Ubiquitination analysis of TRIM24 13. Dual-Luciferase reporter assay 14. Co-immunoprecipitation (Co-IP) 15. RNA immunoprecipitation (RIP) 16. Chromatin immunoprecipitation (ChIP) 17. A xenograft mouse model of NPC 18. Statistical analysis	• Molecules such as miR-106a-5p, TRIM24, and SRGN were shown to be upregulated in both NPC tissues and cells, but FBXW7 was downregulated • Exosomes containing miR-106a-5p were able to enter NPC cells. High levels of miR-106a-5p appeared to enhance the proliferation, migration, invasion, and metastasis of cancer cells. Scientists saw a decrease in aggressive behaviors when they lowered the amounts of this miRNA • miR-106a-5p targets FBXW7, a protein that regulates the levels of TRIM24, another factor in this cancer scenario. TRIM24 influences SRGN, a protein crucial for cancer development • Researchers successfully inhibited miR-106a-5p in exosomes, decelerating the development and metastasis of NPC in live creatures during studies. It appears that a small chemical, transported by exosomes, has a significant role in increasing the aggressiveness of NPC	Exosomal miR-106a-5p accelerates the progression of NPC by decreasing FBXW7 expression, increasing TRIM24 levels, and elevating SRGN expression. The research illuminates the role of exosomal miR-106a-5p on NPC's proliferation and suggests potential treatment strategies by targeting these pathways

Table 2 (continued)

Author	Year	Country	Type of Study	Cancer type	Tissue sample	Tissue processing technique	Observed role of CAF	Conclusion
Wei-Zhou Wang – Et al [10]	2023	china	In vitro (human) and In vivo (animal)	oral squamous cell carcinoma (OSCC)	In vitro case sample: 1. hOMF (CellResearchCorp, Singapore) 2. Cal-27 (Cellcook, Guangzhou, China) In vivo case sample: BALB/c-Nude mice were purchased from the Animal Experiment Center of Kunming Medical University	1. Induction of CAFs and extraction of CAFs-Exo 2. Cal-27 cells were co-cultured with CAFs-Exo or hOMF-Exo 3. Tumor-forming and exosomes injection in nude mice 4. Protein extraction and western blotting 5. Detection of Cal-27 and exosome genes with quantitative PCR assay 6. Sequencing and raw data processing 7. Identification of immune-associated genes 8. PPI network construction and module analysis 9. Screening of candidate genes related to immune cells 10. mRNA-miRNA interaction network construction by WGCNA 11. Development of immune-related signature 12. Analysis of CAFs-Exo target genes by miRNA-miRNA interaction network 13. Statistical analysis	• hsa-miR-139-5p and ACTR2 found in CAFs-Exo may have increased the amounts of CD81 and PIGR in Cal-27 cells, thereby clarifying CAFs-Exo's role in enhancing OSCC proliferation • CAFs-Exo has a potent capacity to promote OSCC proliferation and are linked to immunological suppression. Through the analysis of sequencing data from CAFs-Exo and publicly accessible TCGA data, it was found that immune-related genes in CAFs-Exo can impact the expression of PIGR, CD81, UACA, and PTTG1IP in Cal-27 cells. CAF-derived exosomes may be involved in immunological regulation and promoting the formation of oral squamous cell carcinoma	CAF-s-Exo is involved in controlling tumor immunity through hsa-miR-139-5p, ACTR2, and EIF6. PIGR, CD81, UACA, and PTTG1IP are potential targets for future OSCC therapies

PRISMA 2020 flow diagram for new systematic reviews which included searches of databases

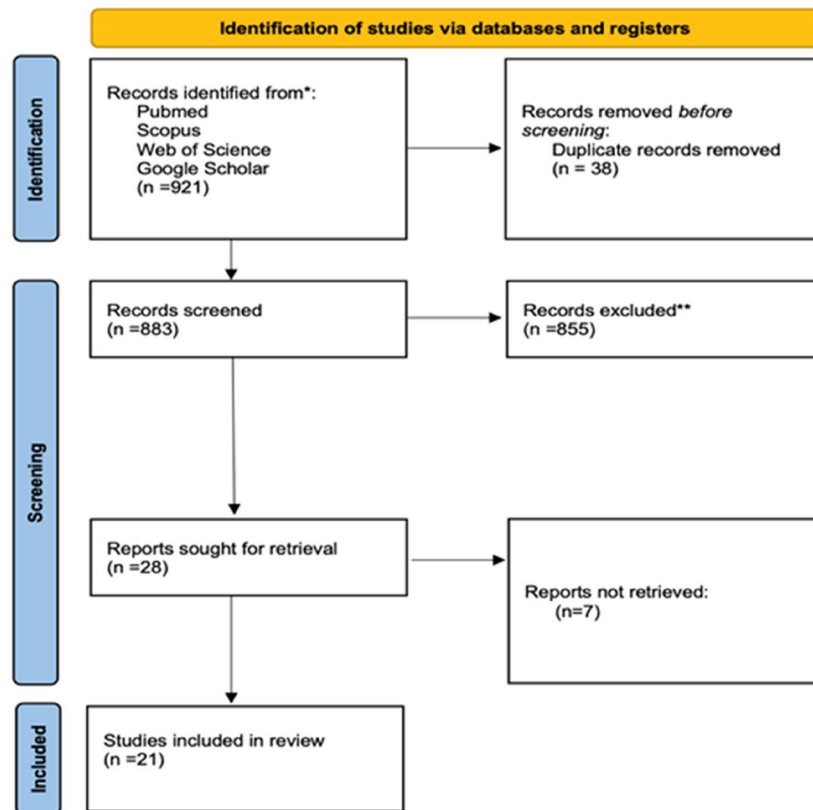


Fig. 1 Flow diagram of study selection procedure

as the most accurate in differentiating between superficial and deep tumors in a separate research study. This mechanism was associated with decreased miR-1-3p levels and increased miR-21-5p expression. Patients with higher levels of miR-133a-3p tended to have better overall survival and remained disease-free for longer periods. [23]. Moreover, having high levels of miR-106-5p and low levels of miR-320a and miR-222-3p were indications of potential malignancy [6]. In an in vivo study, injecting miR-124 into mice's bloodstream slowed the growth of oral tumors [11]. miR-34a-5p overexpression in CAFs in xenograft experiments can hinder the development of OSCC cells by interacting with its immediate downstream target, AXL, hence reducing OSCC cell growth and spread. Recent findings indicate that a type of microRNA called hsa-miR-139-5p, which comes from cancer-associated fibroblast exosomes, might boost the levels of CD81 and PIGR expression in Cal-27 cells, contributing to CAFs-Exo's enhanced capacity to stimulate OSCC proliferation and induce immunosuppression. Min et al. demonstrated that increasing the levels of miR-148a in cancer-associated fibroblasts (CAFs) notably hindered the movement and penetration of oral carcinoma cells

(SCC-25) by specifically affecting WNT10B [14]. A different research found that exosomes generated from cancer-associated fibroblasts carry miR-382-5p, which, when overexpressed, led to the migration and invasion of oral squamous cell carcinoma cells [1]. miR-675-5p/PFKFB3 play a vital role in the lncRNA H19-controlled glycolysis process in oral cancer-associated fibroblasts (CAFs). This interaction may function as a novel biomarker for molecular diagnosis of oral squamous cell carcinoma (OSCC) and as a focal point for anti-tumor therapies [2]. MiR-204 is a microRNA that is reduced in cancer-associated fibroblasts (CAFs) and functions as a tumor suppressor by blocking fibroblast movement. It accomplishes this by controlling the expression of several molecules and specifically focusing on ITGA11 [16].

Esophageal squamous cell carcinoma (ESCC)

The study of the role of miRNAs on esophageal squamous cell carcinoma (ESCC) included a review of 5 articles [3, 5, 7, 18, 21]. CAFs secrete exosomes with miR-21 that are transmitted to monocytes. Exosomal miR-21 is released into the cell's cytoplasm, where it targets PTEN. Zhao and his team proposed that miR-21 switches monocytes

into M-MDSCs by blocking PTEN, which then triggers the activation of STAT3 signaling. Esophageal squamous cell carcinoma patients with CAF-induced M-MDSCs showed cisplatin resistance and poorer outcomes, underscoring the critical role of CAFs working with M-MDSCs to increase treatment resistance [3]. A study on patients who received neoadjuvant chemotherapy found that miR-27a/b plays a role in chemotherapy resistance in esophageal cancer by transforming normal fibroblasts into cancer-associated fibroblasts [18]. In 2017, Khazaei et al. proposed that cancer-associated fibroblasts utilize exosomal miR-451 as a signaling molecule to create a conducive environment for tumor cell migration and cancer advancement [7]. Jin et al. recently compared tumor cell development in laboratory conditions and living organisms. They discovered that the presence of miR-3656 significantly enhanced the proliferation, migration, and invasion abilities of ESCC cells. This indicates that miR-3656-containing exosomes originating from CAFs play a role in the malignant advancement of ESCC [21]. Another study demonstrated that miR-100-5p levels in exosomes obtained from cancer-associated fibroblasts were considerably lower than those derived from normal fibroblasts. miR-100-5p suppressed the growth, movement, penetration, and tube creation in tumor-associated lymphatic endothelial cells (TLECs). In an in vivo setting, miR-100-5p suppressed lymphangiogenesis in ESCC xenografts [5]. An independent research project examined 18 miRNAs and observed significant upregulation or downregulation of microRNAs in the conditioned media of a co-culture system. Research suggests that pathways involving cell adhesion, endocytosis, and cell junctions may be linked to the characteristics of cancer-associated fibroblasts (CAFs) and the advancement of tumors. High amounts of miR-33a and miR-326 were seen in the exosomes recovered from co-cultured and untreated conditioned media. Their study offered valuable information on how cancer-associated fibroblasts (CAFs) work by releasing miRNAs in the tumor microenvironment, indicating potential novel therapy targets for esophageal cancer and possibly other types of cancer [15].

Head and neck squamous cell carcinomas (HNSCC)

Among the studies that investigated the role of miRNAs in HNSCC [12, 19], it was found that both miR-196a and miR-196b elicit cell-specific responses in target genes and downstream regulatory pathways and have a distinctive impact on cell proliferation, migration, and invasion. Álvarez-Teijeiro et al.'s studies indicate that miR-196b dysregulation occurs early and is common in HNSCC carcinogenesis, indicating its potential for early detection and disease status monitoring [12]. HNSCC cells were

discovered to induce CAF-like differentiation by releasing TGF- β and small extracellular vesicles (sEVs) with increased miR-192/215 family miRNAs. Fibroblasts take in tiny extracellular vesicles enriched with miR-192/215, decreasing Caveolin-1 (CAV1) levels and activating TGF- β signaling. Cancer cells can trigger the conversion of stromal fibroblasts into cancer-associated fibroblasts (CAFs), promoting tumor progression through a feedback mechanism [19].

Supraglottic laryngeal squamous cell carcinoma (SLSCC)

Wu et al. studied tumor samples from SLSCC patients and discovered that miR-656-3p, miR-337-5p, miR-29a-3p, and miR-655-3p were decreased, while miR-184-3p, miR-92a-1-5p, miR-212-3p, and miR-3135b were increased. The top five miRNAs in the interaction network are miR-16-5p, miR-29a-3p, miR-34c-5p, miR-32-5p, and miR-490-5p. The top five target genes are CCND1, CDKN1B, CDK6, PTEN, and FOS. The top five miRNAs and their target genes could jointly create a malignant tumor microenvironment and act as markers for SLSCC treatment [24].

In the Supraglottic Laryngeal Squamous Cell Carcinoma (SLSCC) section, we identified several key dysregulated miRNAs that play a crucial role in the pathogenesis of this cancer. Among them, the top five miRNAs include miR-16-5p, miR-29a-3p, miR-34c-5p, miR-32-5p, and miR-490-5p. These miRNAs were found to interact with critical target genes, contributing to tumor progression and the creation of a malignant tumor microenvironment [24, 25].

miR-16-5p miR-16-5p has been extensively studied in various cancers, including breast cancer and lung cancer, where it typically functions as a tumor suppressor. In SLSCC, miR-16-5p downregulates the expression of CCND1 (Cyclin D1), a gene associated with cell cycle regulation, leading to cell cycle arrest and inhibition of tumor growth. Similar findings in other cancers support its role in controlling cell proliferation and tumorigenesis-29a-3p:

This miRNA is involved in regulating epigenetic modification through the repression of DNA methyltransferases and histone deacetylases. In SLSCC, miR-29a-3p was shown to decrease the expression of CDKN1B (p27), a cyclin-dependent kinase inhibitor that regulates the cell cycle. In lung cancer and colorectal cancer, miR-29a-3p has been associated with increased tumor cell migration and invasion through its regulation of similar targets [26, 27].

miR-34c-5p Is well-documented as a regulator of tumor suppression via the p53 pathway. In SLSCC, miR-34c-5p targets CDK6 (Cyclin-dependent kinase 6), which promotes the progression of the cell cycle. The downregulation of CDK6 by miR-34c-5p leads to reduced tumor cell proliferation, a mechanism mirrored in other cancers like ovarian cancer and breast cancer, where it also acts as a tumor suppressor.

miR-32-5p In regulating apoptosis and cell survival, miR-32-5p in SLSCC targets PTEN (Phosphatase and Tensin Homolog), a tumor suppressor gene involved in regulating the PI3K/AKT signaling pathway. miR-32-5p has been shown to reduce PTEN expression, thereby enhancing cancer cell survival and contributing to chemoresistance, similar to its role in glioblastoma and breast cancer [28].

miR-490-5p miR-490-5p is a regulator in several cancers, including ovarian and prostate cancer, where it functions to modulate FOS (a gene encoding a transcription factor involved in cell proliferation and survival). In SLSCC, miR-490-5p targets FOS, promoting tumor cell migration and invasion. This mechanism aligns with findings in other cancers, where miR-490-5p is implicated in tumor progression and metastasis.

The dysregulated expression of RNAs in SLSCC contributes to a complex network of tumor-promoting mechanisms, including the regulation of the cell cycle, apoptosis, and tumor metastasis. The interaction between these miRNAs and their target genes, such as CCND1, CDKN1B, CDK6, PTEN, and FOS, plays a pivotal role in shaping the malignant tumor microenvironment and advancing the progression of SLSCC. These findings underscore the potential of miRNAs as diagnostic markers and therapeutic targets for improving treatment strategies in SLSCC. Additionally, similar mechanisms have been observed in other cancers, highlighting the broader relevance of these miRNAs across malignancies [29, 30].

Nasopharyngeal carcinoma (NPC)

Li et al. conducted a recent investigation and found that exosomal miR-106a-5p can enter NPC cells. Increased expression of this miRNA promoted the growth, movement, infiltration, and spread of NPC cells, which were counteracted by decreasing the levels of exosomal miR-106a-5p. Exosomal miR-106a-5p facilitated the progression of nasopharyngeal carcinoma by reducing FBXW7 levels and increasing

TRIM24 and SRGN levels via FBXW-mediated TRIM24 breakdown [22].

Head and neck cancer (HNC)

Two articles focused on head and neck cancer showed the role of miR-7 and miR-196a [4, 20]. Increased expression of miR-7 was found in cancer-associated fibroblasts (CAFs). RASSF2 negatively controls the miR-7 target gene, reducing the production of PAR-4 in the tumor microenvironment [4]. Reports indicate that exosomal miR-196a from cancer-associated fibroblasts contributes to cisplatin resistance in head and neck cancer via affecting CDKN1B and ING5. This suggests that miR-196a could be a valuable predictor and target for overcoming cisplatin resistance in head and neck cancer [20].

Discussion

OSCC

Matos et al. [23] and Junior et al. [6] similarly demonstrated how microRNAs interact with the remodeling of the extracellular matrix in oral squamous cell carcinoma (OSCC). Both research used ex vivo models with the same material, procedure, and number of case samples. They both indicated a high expression of miR-21-5p, which proved to be the most accurate in distinguishing between superficial and deep cancers. Matos et al. asserted that the well-documented cancer-causing role of miR-21-5p justifies its increased expression in distinguishing between malignant and healthy tissue in deep samples. Additionally, it has been shown that patients with elevated levels of miR-133a-3p showed better overall and disease-free survival rates. The presence of miR-1-3p was linked to better disease-free survival outcomes. The research discovered that miR-1-3p and miR-133a-3p were expressed at reduced levels in deep specimens compared to superficial tumors, suggesting their potential role in the invasion of oral squamous cell carcinoma (OSCC) [23]. Higher levels of miR-21-5p and miR-106-5p, along with lower levels of miR-320a and miR-222-3p, were indicators of malignancy [6].

The study by Li et al. found that oral cancer cells (OCCs) and cancer-associated fibroblasts (CAFs) had lower levels of miR-124 than normal fibroblasts (NFs) and that oral squamous cell carcinoma (OSCC) tissues had lower levels of miR-124 than adjacent normal tissues. The precise biological roles of miR-124 in the interplay between cancer-associated fibroblasts (CAFs) and ovarian cancer cells (OCCs) had not been studied before. The study showed that reintroducing miR-124 in the co-culture of cancer-associated fibroblasts (CAFs) and oral cancer cells (OCCs) inhibited the CAFs' capacity to enhance the development and movement of OCCs.

On the other hand, blocking miR-124 in NFs-OCCs coculture increased NFs' capacity to promote OCCs' cell proliferation and movement. The results emphasize the critical function of miR-124 in the collaborative relationship between cancer-associated fibroblasts (CAFs) and ovarian cancer cells (OCCs) [11].

A xenograft experiment found that oral squamous cell carcinoma (OSCC) cells displayed a more aggressive behavior when exposed to exosomes from cancer-associated fibroblasts (CAFs) that were deficient in miR-34a-5p. The transfer of miR-34a-5p was discovered to influence the growth and movement of OSCC cells via the AKT/GSK-3 β / β -catenin/Snail signaling pathway. The results indicate that drugs aimed at the miR-34a-5p/AXL pathway could effectively treat oral squamous cell cancer [8].

Recent research indicates that hsa-miR-139-5p and ACTR2 found in exosomes originating from cancer-associated fibroblasts (CAFs-Exo) could increase the levels of CD81 and PIGR in Cal-27 cells, thereby enhancing the distinctive capacity of CAFs-Exo to stimulate the proliferation of oral squamous cell carcinoma (OSCC) [10].

Min et al. examined the miR148a expression in normal fibroblasts (NFs) and cancer-associated fibroblasts (CAFs) isolated from human oral squamous cell carcinoma (OSCC) tumor tissues. Their objective was to determine how differences in miR148a expression between normal fibroblasts (NFs) and cancer-associated fibroblasts (CAFs) affected the movement and penetration of oral cancer cells. The study findings indicated a significant decrease in miR-148a levels in cancer-associated fibroblasts (CAFs) as compared to normal fibroblasts (NFs) in oral squamous cell carcinoma (OSCC). Further, in vitro coculture experiments demonstrated that the reduced expression of miR148a in cancer-associated fibroblasts (CAFs) increased the migratory and invasive properties of SCC-25 cells, a specific type of human oral cancer cells. The results suggest that the expression of miR-148a in cancer-associated fibroblasts (CAFs) can significantly influence the biological processes of oral squamous cell carcinoma (OSCC). The researchers proposed that miR-148a in cancer-associated fibroblasts (CAFs) could serve as a valuable biomarker and a potential therapy target in oral squamous cell carcinoma [14].

In a separate study, researchers discovered a new method via which cancer-associated fibroblasts (CAFs) aid in advancing oral squamous cell carcinoma (OSCC). Researchers discovered that exosomes generated from cancer-associated fibroblasts (CAF) carry a particular microRNA, miR-382-5p, to oral squamous cell carcinoma (OSCC) cells, enhancing their ability to migrate and invade. While miR-382-5p is highly expressed in cancer-associated fibroblasts (CAFs) and can promote aggressive characteristics in oral squamous cell carcinoma (OSCC)

cells, it does not influence the abundance of CAFs in OSCC tissues. miR-382-5p is implicated in the communication between cancer-associated fibroblasts (CAFs) and oral squamous cell carcinoma (OSCC) cells, although it does not affect the total quantity of CAFs. The researchers noted that miR-382-5p does not impact CAF proliferation, which could be why its expression is not linked to CAF density. The results could help identify novel therapeutic targets for cancer treatment [1].

Yang et al. discovered that the long non-coding RNA (lncRNA) H19 significantly affects oral cancer-associated fibroblasts (CAFs). The researchers found that the lncRNA H19 was elevated in oral cancer cell lines and cancer-associated fibroblasts (CAFs). Using small interfering RNA (siRNA) techniques, they demonstrated that decreasing lncRNA H19 impacted the proliferation, migration, and glucose metabolism of oral cancer-associated fibroblasts (CAFs). Suppression of lncRNA H19 led to lower RNA levels of PFKFB3 and miR-675-5p in oral cancer-associated fibroblasts (CAFs). The miR-675-5p/PFKFB3 pathway was identified as a crucial regulator in the glycolysis process influenced by lncRNA H19 in oral cancer-associated fibroblasts (CAFs) [2].

Rajthala et al. discovered that miR-204, a specific microRNA, was significantly decreased in cancer-associated fibroblasts (CAFs) compared to normal oral mucosa. ITGA11 was identified as a specific target of miR-204 by a 3' UTR miRNA target reporter experiment. MiR-204, significantly decreased in cancer-associated fibroblasts (CAFs), functions as a tumor suppressor by inhibiting fibroblast mobility by regulating several pathways and directly targeting ITGA11 [16].

ESCC

The roles of microRNA (miRNA) in esophageal squamous cell carcinoma (ESCC) are examined by reviewing relevant papers [3, 5, 7, 15, 18, 21]. Zhao et al.'s study demonstrated the vital role of miR-21 in exosomes generated by cancer-associated fibroblasts (CAFs). miR-21 is transferred to monocytes, transforming monocytes into myeloid-derived suppressor cells (M-MDSCs) by inhibiting PTEN and enhancing STAT3 activation. CAF-induced myeloid-derived suppressor cells (M-MDSCs) were associated with cisplatin resistance and poorer survival outcomes in individuals diagnosed with esophageal squamous cell carcinoma (ESCC) [3].

A study on neoadjuvant chemotherapy found that miR-27a/b plays a crucial role in chemotherapy resistance by causing normal fibroblasts to convert into cancer-associated fibroblasts (CAFs). Khazaei et al. suggested that cancer-associated fibroblasts (CAFs) use exosomal miR-451 as a signaling molecule to promote tumor cell migration and cancer progression. Jin and colleagues showed

that exosomes carrying miR-3656 from cancer-associated fibroblasts (CAFs) significantly promoted the aggressive development of esophageal squamous cell carcinoma (ESCC) both in laboratory settings and in living organisms [21].

A study found significantly lower levels of miR-100-5p in exosomes produced by cancer-associated fibroblasts compared to those from normal fibroblasts. MiR-100-5p inhibited the proliferation, migration, invasion, and angiogenesis in tumor-associated lymphatic endothelial cells (TLECs). miR-100-5p decreased the development of new lymphatic vessels in esophageal squamous cell carcinoma xenografts in vivo [5].

Researchers found that 18 miRNAs were either significantly increased or decreased in the conditioned media of a co-culture system during a thorough analysis. Pathways related to cell adhesion, endocytosis, and cell junctions displayed enrichment, indicating potential links to the CAF phenotype and tumor progression. High amounts of miR-33a and miR-326 were detected in exosomes derived from cancer cells that were either co-cultured or untreated, suggesting potential novel targets for the treatment of esophageal and potentially other types of cancer [15].

Overall, these investigations highlight the complex role of miRNAs in many aspects of ESCC. The discovered miRNAs and their functional activities lay the groundwork for creating precise therapeutic approaches and innovative interventions in treating esophageal squamous cell cancer [3, 5, 7, 15, 18, 21].

HNSCC

Two studies investigated the diverse functions of microRNAs in Head and neck squamous cell carcinoma. Alvarez-Teijeiro and colleagues demonstrated that miR-196a and miR-196b levels were significantly increased in 95% of head and neck squamous cell carcinoma (HNSCC) tumors, suggesting their probable role in tumor development. Laryngeal dysplasia samples show increased miR-196b expression levels, suggesting a possible role in early regulation of head and neck squamous cell carcinoma (HNSCC) development. No substantial disparities were detected between nonprogressive and progressive dysplasia or subsequent cancers. Both miR-196a and miR-196b are frequently altered in 95–100% of people with Head and Neck Squamous Cell Carcinoma (HNSCC).

Moreover, these miRNAs were detected in saliva samples from both HNSCC patients and healthy controls, with higher concentrations observed in HNSCC patients. This indicates that it can potentially be a minimally intrusive biomarker for early detection and monitoring of health issues. Increased expression of miR-196a/b in cell lines derived from head and neck squamous cell

carcinoma (HNSCC) and cancer-related fibroblasts results in the reduction of specific target genes (ANXA1, HOX family) in both cancer cells (FaDu, UT-SCC-42B) and cancer-associated fibroblasts (CAF) [12].

Zhu et al.'s research indicates that in the low-oxygen environment of head and neck squamous cell carcinoma (HNSCC), tumor cells stimulate the transformation of tumor-associated fibroblasts (CAFs) by utilizing factors found in the low-oxygen environment (CM), including TGF- β and small extracellular vesicles (sEV). Hypoxic sEVs in HNSCC cells, especially in the presence of TGF- β , stimulate MRC-5 fibroblasts to enhance the proliferation, migration, and tumor development of HNSCC cells. The hypoxia small extracellular vesicles (EVs) contain increased levels of miR-192 and miR-215, which regulate the function of HIF-1 α and NF- κ B. Knocking down either miR-192 or miR-215 in tiny extracellular vesicles inhibits the differentiation of cancer-associated fibroblasts, affecting the growth, invasion, and xenograft formation of head and neck squamous cell carcinoma tumors. Hypoxic small extracellular vesicles (EVs) in head and neck squamous cell carcinoma (HNSCC) cells upregulate miR-192/215, leading to the downregulation of fibroblast caveolin-1 (CAV1), an essential gene involved in cancer-associated fibroblast (CAF) formation. One can suppress miR-192/miR-215 or increase CAV1 expression to reduce CAF marker expression in fibroblasts. The findings highlight the critical influence of sEV-derived miRNAs on HNSCC advancement by promoting interaction between tumor cells and fibroblasts in the tumor microenvironment [19].

SLSCC

Wu et al. found a distinct miRNA profile in SLSCC, including decreased levels of miR-656-3p, miR-337-5p, miR-29a-3p, and miR-655-3p, and increased levels of miR-184-3p, miR-92a-1-5p, miR-212-3p, and miR-3135b. The interaction network pinpointed miR-16-5p, miR-29a-3p, miR-34c-5p, miR-32-5p, and miR-490-5p as the primary miRNAs that target CCND1, CDKN1B, CDK6, PTEN, and FOS. These miRNAs may cooperate with their target genes to create a malignant tumor environment in SLSCC. These miRNAs and their targets could be important markers for SLSCC treatment [24].

NPC

Li et al.'s research on NPC focused on the oncogenic role of exosomal miR-106a-5p in promoting the growth, movement, infiltration, and spread of NPC cells in laboratory and living organism settings. Reducing exosomal miR-106a-5p lessened this effect. The strategy involved suppressing FBXW7 expression and FBXW-mediated TRIM24 degradation, leading to increased levels of

TRIM24 and SRGN. Targeting exosomal miR-106a-5p could help slow down the advancement of NPC [25].

HNC

Two studies in the head and neck cancer realm investigated miR-7 and miR-196a. Upregulation of miR-7 in Cancer-Associated Fibroblasts (CAFs) decreased the secretion of PAR-4 in the tumor microenvironment by suppressing RASSF2, the gene regulated by miR-7. Another study discovered that exosomes derived from cancer-associated fibroblasts containing miR-196a contribute to cisplatin resistance in head and neck cancer via influencing CDKN1B and ING5. miR-196a has been recognized as a potential predictor and therapeutic target for combating cisplatin resistance in Head and Neck Cancer [4, 26].

Mechanisms of miRNAs in SLSCC, NPC, and HNC: induction and attenuation of malignancy

In SLSCC, NPC, and HNC, the dysregulation of specific miRNAs contributes to key oncogenic processes such as cell proliferation, migration, invasion, apoptosis resistance, and chemoresistance. These processes are facilitated by miRNAs interacting with target genes that are involved in critical cellular pathways, including cell cycle regulation, apoptosis, and signal transduction.

miRNAs inducing malignancy.

1. miR-21 (Common across SLSCC, NPC, and HNC):

Induction of Malignancy: miR-21 has been frequently found to be upregulated in many cancers, including HNC. It exerts its oncogenic effects by targeting PTEN, a well-known tumor suppressor that negatively regulates the PI3K/AKT pathway. By inhibiting PTEN, miR-21 enhances cell survival, proliferation, and migration while promoting resistance to chemotherapy. This mechanism is consistent across various cancers, such as breast cancer and glioblastoma [31].

Target Genes: In HNC, miR-21 targets PDCD4 (Programmed Cell Death 4), which is involved in promoting apoptosis. By downregulating PDCD4, miR-21 reduces apoptosis, allowing cancer cells to survive under conditions of stress, such as during chemotherapy or radiation therapy [32].

2. miR-196a (SLSCC and NPC):

Induction of Malignancy: miR-196a is known to promote epithelial-to-mesenchymal transition (EMT), a key process that contributes to metastasis in several cancers. In SLSCC and NPC, miR-196a targets HOXA10, a gene that regulates cellular localization

and movement during development. The overexpression of miR-196a leads to the activation of HOXA10, facilitating tumor invasion and metastasis.

Target Genes: miR-196a's regulation of HOXA10 suggests a mechanism through which miR-196a promotes local tumor growth and distant metastasis, a hallmark of aggressive cancers like NPC [33].

3. miR-34c-5p (SLSCC):

Induction of Malignancy: miR-34c-5p acts as a tumor suppressor in SLSCC but can also promote malignancy in certain contexts by downregulating CDK6 (Cyclin-Dependent Kinase 6). This gene is crucial for cell cycle progression and is often overexpressed in cancer cells. By targeting CDK6, miR-34c-5p inhibits cell cycle progression, ultimately promoting apoptosis and preventing uncontrolled cell growth in healthy cells. In cancer, loss of miR-34c-5p expression could lead to unchecked cell proliferation, contributing to malignancy.

Target Genes: The downregulation of CDK6 prevents the activation of cyclin-dependent kinases necessary for the G1/S transition of the cell cycle, reducing tumor proliferation in SLSCC.

4. miR-29a-3p (SLSCC, NPC):

Induction of Malignancy: miR-29a-3p targets CDKN1B (p27), an inhibitor of cyclin-dependent kinases, promoting cell cycle progression. In NPC and SLSCC, high levels of miR-29a-3p are associated with increased cell proliferation and tumor progression.

Target Genes: p27 regulates the transition between the G1 and S phases of the cell cycle. miR-29a-3p-induced downregulation of p27 enhances the G1/S transition, accelerating tumor cell division [34].

miRNAs attenuating malignancy

1. miR-7 (SLSCC and NPC):

Attenuation of Malignancy: miR-7 is often downregulated in cancer, and its restoration has been linked to tumor suppression. In SLSCC and NPC, miR-7 inhibits PI3K/AKT signaling, which is a key pathway promoting survival and proliferation. miR-7 targets RASSF2, a tumor suppressor gene involved in cell cycle arrest and apoptosis. By restoring miR-7 expression, inhibiting PI3K/AKT signaling and promoting apoptosis may reduce tumor growth and increase chemosensitivity.

Target Genes: RASSF2 regulates apoptosis and cell cycle progression. The downregulation of RASSF2 by miR-7 results in reduced cell survival and proliferation.

tion, attenuating tumor growth in SLSCC and NPC [35].

2. miR-148a (NPC and HNC):

Attenuation of Malignancy: miR-148a has been identified as a tumor suppressor in multiple cancer types, including NPC and HNC. It downregulates DNMT1 (DNA methyltransferase 1), which is responsible for maintaining DNA methylation patterns. In cancers, the inhibition of DNMT1 by miR-148a leads to demethylation of tumor suppressor genes and restoration of their expression. This epigenetic regulation contributes to reduced tumor progression [36].

Target Genes: DNMT1 silences tumor suppressor genes by maintaining DNA methylation at promoter regions. By targeting DNMT1, miR-148a induces the re-expression of silenced tumor suppressors, thereby reducing tumor cell growth and metastasis.

Functional roles of target genes in malignancy

The identified target genes—PTEN, PDCD4, HOXA10, CDK6, p27, RASSF2, DNMT1, and FOS—play critical roles in regulating key oncogenic processes such as cell cycle progression, apoptosis, migration, invasion, and metastasis. For example [37]:

- PTEN (Targeted by miR-21) functions as a negative regulator of the PI3K/AKT pathway, and its loss results in increased cell survival and resistance to apoptosis.
- PDCD4 (Targeted by miR-21) is involved in apoptosis, and its downregulation by miR-21 promotes tumor survival.
- HOXA10 (Targeted by miR-196a) is a transcription factor involved in EMT and metastasis. Its overexpression drives tumor invasion and migration.
- CDK6 (Targeted by miR-34c-5p) regulates the G1/S transition of the cell cycle. By inhibiting CDK6, miR-34c-5p prevents uncontrolled cell proliferation.
- p27 (Targeted by miR-29a-3p) inhibits cyclin-dependent kinases and controls cell cycle progression. Its downregulation by miR-29a-3p accelerates the G1/S transition, facilitating tumor growth.
- RASSF2 (Targeted by miR-7) plays a key role in apoptosis and cell cycle regulation. Its inhibition by miR-7 results in reduced tumor cell survival.
- DNMT1 (Targeted by miR-148a) is involved in DNA methylation and gene silencing. Its downregulation by miR-148a promotes the re-expression of tumor suppressor genes, attenuating tumor growth.

Presence of miRNAs in other types of cancer

Several of the miRNAs identified in our review, such as miR-21, miR-196a, miR-7, and miR-148a, are not exclusive to HNSCC but have been implicated in a wide range of other malignancies, including breast cancer, lung cancer, esophageal cancer, and colorectal cancer. For instance:

- miR-21 is frequently upregulated in a variety of cancers, including breast cancer, lung cancer, glioblastoma, and colorectal cancer. In these cancers, miR-21 has been shown to regulate key pathways involved in tumor progression, such as the PI3K/AKT and MAPK pathways, and is often associated with poor prognosis due to its role in inhibiting tumor suppressor genes (e.g., PTEN).
- miR-196a has been reported in gastric cancer, lung cancer, and pancreatic cancer, where it is often involved in regulating oncogenes like HOXA10 and plays a role in the epithelial-mesenchymal transition (EMT), a process that contributes to tumor metastasis.
- miR-7 has been found to be downregulated in several cancers, including breast cancer and prostate cancer, and is known to regulate key signaling pathways such as the PI3K/AKT pathway and interactions with the tumor suppressor gene PAR-4.
- miR-148a has been implicated in liver cancer, breast cancer, and pancreatic cancer. In some contexts, it functions as a tumor suppressor and has been shown to regulate genes involved in cell proliferation, migration, and apoptosis.

Mechanisms of miRNA function across cancers

The mechanisms by which these miRNAs exert their function can be similar across different cancers, as many of the key molecular pathways involved in tumorigenesis are conserved. For example, miRNAs like miR-21 and miR-196a often regulate cell survival, proliferation, and migration genes, which are critical processes in cancer progression. However, the specific target genes and pathways influenced by these miRNAs can vary depending on the cancer type and the specific tumor microenvironment. For instance:

- In HNSCC, miR-21 promotes cancer cell proliferation by targeting tumor suppressor genes such as PTEN and PDCD4, which results in enhanced cell survival and resistance to apoptosis. Similarly, miR-196a modulates the HOXA gene family and is involved in EMT, which facilitates metastasis.

- In breast cancer, miR-21 targets PTEN and Reck, promoting epithelial-to-mesenchymal transition (EMT) and contributing to metastasis. In contrast, HNSCC may also regulate immune escape mechanisms, contributing to chemoradiotherapy resistance.

While the molecular mechanisms are often similar across cancers, the tissue-specific context and the stroma-cancer interactions can affect how these miRNAs function. For example, in HNSCC, miR-7 may target RASSF2 to modulate the PAR-4 pathway, while in breast cancer, it may regulate the PI3K/AKT pathway to suppress cell migration and invasion [38–40].

Similar or opposite functions depending on cancer type?

Indeed, the function of miRNAs can differ depending on the cancer type, and they may act as either oncogenes or tumor suppressors. For example:

- miR-21 is typically an oncogene in most cancers, promoting tumor progression by inhibiting tumor suppressor genes and enhancing cell survival. However, miR-21 also plays a role in resistance to chemotherapy and radiotherapy across various cancers, not just in HNSCC [41, 42].
- miR-7, which is generally downregulated in many cancers and acts as a tumor suppressor, may have a similar role in HNSCC, where it inhibits cancer cell proliferation and migration. However, miR-7 may function differently in some cancers due to tissue-specific factors or mutations in its target genes.
- miR-148a, which often functions as a tumor suppressor in cancers such as pancreatic and breast cancer, has been shown to inhibit cell migration and invasion. In HNSCC, however, its role could be more complex, depending on the specific molecular context.
- miR-196a can act as an oncogene in gastric cancer and lung cancer, promoting cell migration and invasion by regulating HOX genes, while in HNSCC, it may play a more prominent role in promoting tumor stem cell-like properties and therapy resistance.

The miRNAs identified in our review, such as miR-21, miR-196a, miR-7, and miR-148a, are not restricted to HNSCC but also play critical roles in other types of cancer. While the mechanisms by which they exert their functions are often similar across cancers—regulating key pathways like cell survival, proliferation, migration, and invasion—the specific roles of these miRNAs can vary depending on the cancer type and microenvironment. Furthermore, depending on the cancer, these miRNAs

may function as either oncogenes or tumor suppressors, highlighting the complexity of their involvement in cancer biology. Therefore, understanding the precise context and molecular interactions in different cancer types is crucial for harnessing the potential of these miRNAs as diagnostic biomarkers or therapeutic targets [43, 44].

Future research directions:

1. Exploring CAF-Derived miRNAs in other malignancies:

Future studies could investigate the role of CAF-derived miRNAs in other cancer types beyond head and neck malignancies. Exploring their involvement in different tumor microenvironments, such as in breast, lung, or colorectal cancers, could reveal broader insights into the cancer-stromal interactions and their implications for metastasis and treatment resistance [45].

2. Longitudinal studies on CAF-Derived miRNAs as predictive biomarkers:

Longitudinal cohort studies should be conducted to assess how CAF-derived miRNAs change over time and correlate with disease progression, metastasis, and patient outcomes. Such studies could establish miRNAs as dynamic biomarkers that provide real-time insights into tumor evolution and therapeutic responses, aiding in personalized treatment strategies [46].

3. Development of miRNA-based therapeutic strategies:

Investigating the feasibility of using miRNA mimics or inhibitors to modify CAF activity could lead to novel therapeutic approaches. Clinical trials could explore the potential of restoring tumor-suppressive miRNAs or inhibiting oncogenic miRNAs within the CAFs to disrupt tumor progression, metastasis, or resistance to therapies like chemotherapy or immunotherapy.

4. Investigating exosomal miRNA signaling pathways:

Research should delve deeper into the molecular mechanisms by which exosomal miRNAs from CAFs influence the tumor microenvironment. Studies could focus on identifying the precise target genes of these miRNAs and their involvement in regulating immune cell infiltration, angiogenesis, and cancer cell resistance to treatments such as chemotherapy and radiotherapy [47].

5. Integration of multi-omics approaches:

Combining miRNA profiling with other omics technologies, such as proteomics, transcriptomics, and metabolomics, could provide a more comprehensive

understanding of how CAF-derived miRNAs influence the tumor microenvironment. This could lead to identifying novel biomarkers and therapeutic targets, enabling more precise treatment regimens.

Clinical applications:

1. miRNA-based diagnostic tools:

The findings suggest that CAF-derived miRNAs, such as miR-196a and miR-7, could be developed into non-invasive diagnostic tools, potentially detectable in blood, saliva, or exosomes. Such tools would enable early detection, particularly in head and neck cancers, where timely diagnosis often leads to improved survival rates.

2. Targeting miRNAs to overcome chemotherapy resistance:

Given the role of CAF-derived miRNAs, such as miR-196a, in inducing chemotherapy resistance, targeted therapies aimed at modulating these miRNAs could improve treatment efficacy. For example, interventions that block miR-196a could restore sensitivity to chemotherapy agents like cisplatin, offering a promising strategy for patients with resistant head and neck cancers [48].

3. CAF-targeted therapies:

Targeting the CAFs rather than just the tumor cells could be an effective strategy. By targeting CAF-derived exosomes or modulating CAF differentiation through specific miRNA manipulation, it might be possible to disrupt tumor-promoting signals and inhibit metastasis. Clinical trials should explore therapies targeting the CAF-miRNA axis to slow cancer progression and improve outcomes for patients with advanced or metastatic cancers.

4. Personalized medicine:

Integrating CAF-derived miRNA profiling into clinical practice could lead to personalized medicine approaches. By tailoring treatments based on the specific miRNA profiles of patients' tumors and their surrounding fibroblasts, clinicians could optimize therapy regimens, minimizing side effects while maximizing therapeutic efficacy [49].

5. Monitoring disease progression and response to treatment:

miRNAs from CAFs could be utilized to monitor disease progression and patient response to treatment, offering a non-invasive method to track the effectiveness of ongoing therapies. For instance, miR-21, miR-148a, and other key miRNAs could be assessed periodically to adjust treatment plans accordingly [49].

Limitations

This study has several limitations that must be considered when interpreting the findings. First, the heterogeneity among the included studies presents a significant challenge. The studies varied widely in terms of study design, sample size, miRNA extraction, analysis methods, and the types of head and neck cancers studied. This diversity makes it difficult to draw direct comparisons across studies and may limit the generalizability of the findings. Although statistical methods such as the I^2 statistic were employed to assess heterogeneity, the variation across studies still affects the robustness and reliability of the pooled results.

Second, many of the included studies have a moderate to high risk of bias. While we used the Scirap tool for quality assessment, some studies did not provide sufficient details on key methodological aspects such as randomization, blinding, and sample selection. This lack of transparency increases the potential for bias, which could influence the outcomes and affect the overall validity of the conclusions drawn from the review.

Another limitation lies in the lack of standardized protocols across studies. Different approaches to miRNA extraction, profiling, and analysis were used, making it difficult to establish uniform methods for evaluating miRNA expression in head and neck cancers. This inconsistency in laboratory techniques limits the reproducibility of findings and complicates efforts to develop standardized practices for miRNA-related cancer diagnostics or therapies.

The review also predominantly focuses on oral squamous cell carcinoma (OSCC), with limited studies on other head and neck cancer subtypes, such as nasopharyngeal carcinoma (NPC) or esophageal squamous cell carcinoma (ESCC). As a result, the findings may be more reflective of OSCC and may not fully represent the miRNA profiles or mechanisms involved in other types of head and neck malignancies. This narrowing of focus reduces the broader applicability of the results to the entire spectrum of head and neck cancers.

Publication bias is another potential limitation of this review. Like most systematic reviews, we may have unintentionally excluded studies with negative or non-significant results, which are less likely to be published. This could lead to an overestimation of the effectiveness or importance of CAF-derived miRNAs in cancer progression and treatment [50].

Furthermore, while the review highlights the potential of miRNAs as diagnostic biomarkers and therapeutic targets, there is limited clinical data to support these applications. Most of the included studies are preclinical or involve cell-line models, with few directly translating to clinical settings. As a result, the clinical

relevance of miRNA-based diagnostic or therapeutic strategies remains uncertain and warrants further investigation in human clinical trials.

Additionally, many of the included studies did not provide detailed information on patient demographics or other potential confounding variables, such as cancer stage, treatment history, or comorbidities. These factors could influence miRNA expression and affect the interpretation of the results. The omission of such data introduces the possibility of confounding, which weakens the conclusions drawn from the review [51].

Lastly, this study focused primarily on miRNAs and exosomes as key mediators of CAF-cancer cell interactions. However, many other factors, such as cytokines, growth factors, and extracellular matrix components, influence the tumor microenvironment. By not considering these additional modulators, this review may oversimplify the complex interactions between CAFs and cancer cells, limiting our understanding of the full range of mechanisms involved in tumor progression.

Despite these limitations, this study provides valuable insights into the role of CAF-derived miRNAs in head and neck malignancies. However, further research is necessary to validate these findings, address the identified limitations, and explore the clinical relevance of CAF-derived miRNAs in cancer diagnosis and therapy.

Conclusion

In this systematic review, we have explored the role of various dysregulated miRNAs in the pathogenesis of Supraglottic Laryngeal Squamous Cell Carcinoma (SLSCC), Nasopharyngeal Carcinoma (NPC), and Oral Squamous Cell Carcinoma (OSCC), highlighting the complex interactions between miRNAs and their target genes in tumor progression. Our findings underscore the pivotal role of specific miRNAs, such as miR-21, miR-196a, miR-7, and miR-675-5p, in regulating key pathways involved in cell cycle progression, apoptosis, migration, and metastasis. These miRNAs act as key modulators of cancer cell proliferation, resistance to chemotherapy, and the epithelial-to-mesenchymal transition (EMT), which are crucial for tumor aggressiveness and metastasis.

Moreover, the regulation of miRNAs by long non-coding RNAs like H19 in OSCC presents an additional layer of complexity, influencing both the epigenetic landscape and functional pathways crucial for cancer progression. The integration of miRNA-based therapeutic strategies, such as targeting the H19/miR-675-5p axis, holds promise for improving treatment outcomes and overcoming resistance to conventional therapies.

Given the potential of miRNAs as diagnostic biomarkers and therapeutic targets, further research is essential to validate these findings and explore the clinical

applicability of miRNA-based interventions. Continued investigations into the molecular mechanisms underlying miRNA dysregulation in head and neck cancers will pave the way for more personalized and effective treatment strategies, ultimately improving patient prognosis and survival.

In conclusion, this review highlights the importance of miRNAs in the molecular landscape of SLSCC, NPC, and OSCC and their potential as valuable tools for enhancing early detection, guiding treatment decisions, and developing targeted therapies in the future.

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Competing interests

The authors declare no competing interests.

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