Cancer Epidemiology 58 (2019) 137–145

Contents lists available at ScienceDirect

Cancer Epidemiology

journal homepage: www.elsevier.com/locate/canep

Liquid biopsy: miRNA as a potential biomarker in oral cancer

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ARTICLE INFO

Keywords:
Oral squamous cell carcinoma
Non-invasive biomarker
Liquid biopsy
Cell free nucleic acid
Circulating microRNA

ABSTRACT

Oral cancer is one of the leading cancers in South-Asian countries. Despite the easy access of the oral cavity, the detection and five year survival rates of OSCC patients are dismal. Identification of non-invasive biomarkers to determine the progression and recurrence of OSCC could be of immense help to patients. Recent studies on oral cancer suggest the importance of non-invasive biomarker development. Micro-RNAs (miRNAs) are one of the important components of the cell-free nucleic acids available in different body fluids. Here, we have reviewed the current understanding of circulating miRNAs as non-invasive biomarkers in different body fluids of oral cancer patients. A number of circulating miRNAs are found to be common in the body fluids of OSCC patients, while many of these are study specific, the possible sources of this variability could be due to differences in sample processing, assay procedure, clinical stage of the disease, oral habit and environmental factors. The prognostic and therapeutic significance of these circulating miRNAs are suggested by several studies. Mir-371, mir-150, mir-21 and mir-7d were found to be potential prognostic markers, while mir-134, mir-146a, mir-338 and mir-371 were associated with metastases. The prognostic markers, mir-21 and mir-7d were also found to be significantly correlated with resistance to chemotherapy, while mir-375, mir-196 and mir-125b were significantly correlated with sensitivity to radiotherapy. Despite the promising roles of circulating miRNAs, challenges still remain in unravelling the exact regulation of these miRNAs before using them for targeted therapy.

1. Introduction

Head and neck squamous cell carcinoma (HNSCC), the ninth most common neoplasm across the globe, is a significant cause of cancer morbidity and mortality [1]. HNSCC mainly comprises neoplasms of the oral cavity, pharynx, larynx, paranasal synapses, nasal cavity and salivary glands. According to the recent GLOBOCAN 2018 report, cancers of the lip and oral cavity are the most frequent cancer in Melanesia and South Central Asia [2]. It is the leading cause of cancer death among men in India and Sri Lanka [2]. Oral squamous cell carcinoma (OSCC) is the major component of HNSCC, accounting for about 90% of all oral malignancies representing a major global burden with an estimated 300,373 cases per annum [1]. The recent GLOBOCAN 2018 report estimated 354,864 new cases of lip and oral cavity cancer per annum with around 1.8 million deaths predicted in 2018 [2]. Oral cancer consists of malignant neoplasms arising in the lips, hard palate, upper and lower alveolar ridges, sublingual region, buccal mucosa, anterior two-thirds of the tongue, retromolar trigone and floor of the mouth [3,4]. Variations in the incidence of oral cancer across the globe are generally attributed to differences in habits practised by different populations [1]. The habits of smoking, chewing tobacco, areca nut, betel quid, alcohol consumption, poor oral hygiene, chronic mechanical trauma and Human Papillomavirus (HPV) infections are considered to be the major factors for the higher incidence of OSCC in India [3,5]. Oral potentially malignant disorders (OPMD) are also reported to advance to malignancies, if left untreated [5].

One of the main reason for poor survival of oral cancer patients is late detection. Clinical examination of the oral cavity and biopsy of the suspected lesion followed by histological analysis are generally used for diagnosis. Biopsy is an invasive procedure in which a portion of the suspected malignant tissue is obtained and further subjected to specialized and sophisticated histopathology or cytology procedures. Biopsy is gold standard till date in detecting the histopathological type of a neoplasms and its degree of differentiation, and has been in practice since the 11th century [6,7]. Sometimes it becomes difficult to procure biopsy material due to inaccessibility of certain tumors, physical pain involved after the procedure, surgical complications, financial burden and lack of trained clinicians. Cell free nucleic acids

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https://doi.org/10.1016/j.canep.2018.12.008
Received 14 June 2018; Received in revised form 6 December 2018; Accepted 12 December 2018
Available online 19 December 2018
1877-7821/

(cfNAs), such as cell free DNA and RNA are present in body fluids of all individuals [8,9]. The expression profile of cfNAs varies between different disease states, so liquid biopsy has potential as a minimally invasive method for detection of disease, including malignant neoplasms [10,11].

1.1. Circulating MicroRNAs as liquid biopsy biomarkers in cancer

Liquid biopsy first came on the scene in 1984 when Mandel and Metais referred to cfNAs as free floating nucleic acids in blood [8,9]. The source of cfNA in body fluids of cancer patients is considered to be apoptotic and necrotic cells of the neoplasm itself, and of other cells in the tumour microenvironment. Secretion has also been suggested as a potential source of cfNA [8]. Circulating tumor cells were first observed in the blood of a cancer patient in 1869, but it took more than a century before their entry into commerce. The clinical utility of liquid biopsy was first realized in 1994 when point mutations of a gene were successfully detected from the cfDNA derived from the plasma of acute myelogenous leukemia patients, whereas the DNA derived from the cellular component of blood did not show any mutations [12].

MiRNAs are usually secreted into the body fluids in membrane bound vesicles known as exosomes. Exosomes are 50–100 nm-sized membrane bound molecular carriers that play important roles in cell-cell interaction [13]. Release of miRNAs from exosomes is a significant mechanism of genetic exchange between cells [14]. Circulating miRNAs are extremely stable and can be conveniently used as informative biomarkers for complex diseases such as cancers [15]. Recent studies of circulating miRNAs in plasma, serum, and other body fluids show that miRNAs secreted from a particular cell type not only has a local action, but may also act at distant sites [16,17].

2. Serum/plasma miRNA as a biomarker in oral cancer

Circulating miRNAs have been demonstrated to show different levels in the body fluids of OSCC patients compared to healthy individuals [18,19]. MiRNAs are highly stable in the serum or plasma leading to rising hopes for their future use as cancer biomarkers [20]. Circulating miRNAs are not digested by RNase and are stable at high pH, after boiling and after multiple freeze-thaw cycles [21,22]. While bound to the argonaute protein, miRNAs are stable in the extracellular environment whether encapsulated inside the extracellular vesicles such as exosomes or freely circulating [23,24]. Exosome-miRNAs are reported to represent a subset of about 3% of the entire amount of cell-free miRNAs [24].

In the last few years, several miRNAs have been reported to show differential expression levels in the serum or plasma of OSCC patients compared to healthy controls, although there is considerable variation in the types identified (Table 1). Yan et al. studied the expression of miRNAs in the plasma among OSCC patients from Denmark and China [25]. Next generation sequencing in Danish cohort identified increased levels of miR-26a-5p, miR-148a-3p, miR-21-5p and reduced levels of miR-486-5p [25]. On the contrary, quantitative analysis performed on the Chinese cohort identified lower levels of miR-375, miR-92b-3p and miR-486-5p, but did not show significant changes in the miRNAs that were found to be higher in the plasma of Danish cohort [25]. Since the method of plasma collection and handling of specimens in both cohort was uniform, the differences may be attributed to the population *per se*: to genetic inheritance or exposure to different risk factors for oral cancer. Another study on a Canadian population reported increased levels of 15 miRNAs and reduced levels of 7 miRNAs in the serum of OSCC patients compared to healthy controls [18]. Meanwhile Ayaz et al. reported a different set of miRNAs in the plasma of OSCC patients among a Turkish population [19]. Since both these studies involved a majority of patients having a Caucasian ethnicity, the differences in expression might be due to the sample source or method of sample processing. A detailed list of the expression profiles of miRNAs in the serum or plasma of OSCC patients is presented in Table 1.

Despite the stability of circulating miRNAs and many encouraging results, these miRNAs have not found routine use as cancer biomarkers. Inconsistent results of miRNA profiles in serum and plasma have been reported [26]. Serum has been reported to show higher miRNA concentrations than corresponding plasma, suggesting that the coagulation process may affect the total amount of miRNA present [27]. Other issues relate to differences in sample handling and processing [28]. For example variations in speed and duration of centrifugation may influence the number of platelets and microvesicles remaining in the supernatant, and platelets may in turn unleash their miRNA leading to a variation in total amount of cell free-miRNAs [29]. Thus, whether studies are carried out on blood, plasma or on serum, each procedure should aim at reducing the risk of hemolysis [20]. For example, miR-451 and miR-16 are reported to show higher levels in OSCC serum [18]: both these miRNAs are present at high levels in RBCs, so hemolysis may result in elevated concentrations of these miRNAs in serum [30]. To reduce this risk, it has been suggested to centrifuge samples within 2 h from the time of blood collection [31]. Prolonged storage should also be avoided since that has been demonstrated to decrease the concentration of non-exosomal miRNA [31]. The incubation between sample collection and serum isolation is a critical factor for the stability of cell free nucleic acids. Furthermore, the type of anticoagulant in the collection tube can also affect result [32]. Samples can be screened for hemolysis by visual screening or by spectrophotometric quantification at 415 nm [26]. Exosomal purification before extraction of miRNA would minimize the effect of hemolysis. The volume of sample used was also seen to influence the outcome, as presence of inhibitors might affect synthesis of complementary DNA (cDNA) [33].

Another important challenge is the normalization of miRNA expression data due to lack of an appropriate endogenous control. There is a need to find reliable housekeeping miRNAs in the serum or plasma for normalization. A reliable method for overcoming this challenge is introduction of an exogenous miRNA (spike-in-control) in the serum/plasma samples prior to phase separation. The expression of the endogenous miRNAs can be normalized with the expression of the exogenous control, which is spiked in with the samples in equal copy numbers and thus, is expected to show an uniform expression in all samples. However, this strategy does not correct for differences in input RNA quantities while comparing with normal control individuals. Hence, a proper endogenous control is necessary to normalize for the input RNA quantity of the samples due to differences in collection time and handling of the samples. A recent study evaluated the effect of parameters such as gender and fasting on regulating the expression profiles of extracellular circulating miRNAs in healthy individuals [34]. For future cell free miRNA biomarker studies, this could indeed serve as a good reference dataset of healthy individuals.

3. Salivary miRNA as a biomarker in oral cancer

“Whole mouth fluid”(WMF) is a complex biological fluid containing saliva secreted by three paired major salivary glands, namely parotid, submandibular and sublingual, plus a large number of widely distributed minor salivary glands. There is a significant contribution from gingival crevicular fluid, an inflammatory serum and cellular exudates [35]. Moreover, WMF contains serum and desquamated epithelial cells from the oral mucosa, including some leucocytes, especially if there is ulceration or mucositis, the latter being inevitable if a neoplasm is present [35,36]. Since collection and processing of WMF is simple, relatively noninvasive and cost effective, it has been used extensively to extract meaningful biological data in different localized and systemic disorders [37,38]. Salivary miRNA biomarkers have recently become an emerging field for monitoring both oral and systemic diseases [37–40]. In carcinogenesis, overexpression of certain miRNAs could result in downregulation of tumor suppressor genes, while reduced expression of certain miRNAs could cause oncogene upregulation [41]. Therefore,

Table 1
miRNAs reported to show different levels in serum/plasma and their functional significance.

miRNA	Sample	miRNA level in OSCC vs. Normal	Demography	Functional role
miR-16	Serum	High No change	Canadian [18] Dutch [80]	Tumor suppressors downregulating oncogenes like RAS and BCL2 [79]
miR-Let-7b	Serum	High	Canadian [18]	Tumor suppressors downregulating oncogenes like RAS and BCL2 [79]
miR-338	Serum	Low	Canadian [18]	Tumor suppressor Inhibits proliferation and metastasis of OSCC cells by targeting NRP1 [81]
miR-29a	Serum	Low	Canadian [18]	Tumor suppressor Inhibits OSCC cell invasion and anti-apoptosis targeting MMP2 gene [82]
miR-9	Serum	Low	Chinese [83]	Tumor suppressor Inhibits proliferation of OSC targeting CXC chemokine receptor 4 and NFKβ [84]
miR-92a	Serum	High	Canadian [18]	–
miR-30e	Serum	High	Canadian [18]	–
miR-320	Serum	High	Canadian [18]	Tumor suppressor Inhibits migration and invasion in TSCC [85]
miR-7	Serum	High	Canadian [18]	Tumor suppressor Targets IGF1R and RECK [86,87]
miR-25	Serum	High	Canadian [18]	Attenuates proliferation of TSCC [88]
miR-195	Serum	High	Canadian [18]	Tumor suppressor Inhibits proliferation and migration targeting TRIM14 [82,89]
miR-624	Serum	High	Canadian [18]	–
miR-142	Serum	Low	Canadian [18]	Inhibits growth and colony formation targeting TGFBR1 [79,90]
Let-7d	Serum	Low	Canadian [18]	Tumor suppressor Inhibits migration and invasion. Increases chemosensitivity in OSCC [67,72]
miR-181	Plasma	High	Taiwanese [66]	Tumor suppressor Targets p27kip1 and Bcl-2 Enhanced cell migration and invasion [66]
miR-196a/b	Plasma	High	Taiwanese [91]	Oncogene Enhanced invasion, migration and adhesion
miR-331-3p	Plasma	High	Turkish [19]	–
miR-603	Plasma	High	Turkish [19]	–
miR-1303	Plasma	High	Turkish [19]	–
miR-660-5p	Plasma	High	Turkish [19]	–
miR-212-3p	Plasma	High	Turkish [19]	–
miR-99b	Plasma	High	Turkish [19]	Tumor suppressor Inhibits cell proliferation targeting GSK3β in OSCC [92]
miR-194-5p	Plasma	High	Turkish [19]	Tumor suppressor Inhibits cell proliferation by reducing PI3K-Akt-FoxO3a signaling in OSCC and targets Wee1 in LSCC [93,94]
miR-214-3p	Plasma	High	Turkish [19]	Oncogene Promote invasion and migration
miR-335-5p	Plasma	High	Turkish [19]	Generation of a pro-inflammatory SASP by increasing the release of MCP-1, IL-6, and MMP-2, by down-regulating PTEN [95]
miR-18a-5p	Plasma	High	Turkish [19]	–
miR-205-5p	Plasma	High	Turkish [19]	Tumor suppressor Targets ZEB1 and ZEB2 [96,97]
miR-192-5p	Plasma	Low	Canadian [19]	–
miR-150-5p	Plasma	Low	Turkish [18]	Tumor suppressor Inhibits metastasis targeting ZEB1 [57,90]
miR-601	Plasma	Low	Turkish [19]	–
miR-375	Plasma	Low No Change	Chinese [25] Danish [25]	Tumor suppressor Inhibits OSCC cell migration and invasion. Enhances radiosensitivity. Targets PDGF and IGFR [71]
miR-187	plasma	High	Chinese [98]	Oncogene
miR-92b-3p	Plasma	No Change Low	Danish [25] Chinese [25]	–
miR-200b-3p	Plasma	High	Danish [99]	Poor prognosis of OSCC patients
miR-134	Plasma	High	Taiwanese [54]	Oncogene. Induces metastasis in HNSCC Targeting WWOX gene [54]
miR-372	Plasma	High	Chinese [98]	Oncogene Induces nodal metastasis, lymphovascular invasion and poor survival in OSCC. Targets LATS2 [53]
miR-19a	Serum	High	Canadian [18]	Regulation of inflammatory response Targets SOCS3 in OSCC [100]
miR-223	Serum Plasma	Low High	Canadian [18] Japanese [50]	Tumor suppressor Inhibits proliferation and induces apoptosis Targets IGF1R in several cancer [101]
miR-24	Plasma	High	Taiwanese [102]	Oncogene Facilitates growth of OSCC cells by targeting p57
miR-146a	Plasma	High	Chinese [98]	Oncogene Increases tumorigenesis and metastasis in OSCC by targeting IRAK1, TRAF6 and NUMB genes [55]

(*continued on next page*)

Table 1 (*continued*)

miRNA	Sample	miRNA level in OSCC vs. Normal	Demography	Functional role
miR-21	Plasma	High	Japanese [50]	Oncogene
		No change	Chinese [25]	Induces tumor cell invasion and proliferation by targeting PTEN and PCD4 [49]
		High	Danish [25]	
		Low	Turkish [51]	
	Serum	High	Indian [48]	
	Blood	High	German [49]	
miR-184	Plasma	High	Chinese [98]	Oncogene
	Serum	High	Chinese [104]	Antiapoptosis and proliferation of TSCC Targets SF1 [103]
miR-31	Plasma	High	Taiwanese [105]	Oncogene
		No change	Brazilian [97]	miR-31 promotes OSCC by enhancing the migration and invasiveness of OSCC cells targeting ACOX1 [64,106]
miR-27b	Plasma	Low	Taiwanese [52]	Tumor suppressor Inhibits cancer cell migration and invasion targeting MET [107]
miR-148a	Plasma	High	Danish [25]	Tumor suppressor
		No change	Chinese [25]	Inhibits cancer cell migration and invasion targeting Wnt10b
miR-451	Serum	High	Canadian [18]	Tumor suppressor
miR-125b-5p	Plasma	Low	American [43]	Tumor suppressor
		Low	Turkish [51]	Inhibits cell proliferation, migration and invasion targeting HMGA2 [65]
miR-3651	Serum	High	Chinese [108]	–
	Blood	High	German [109]	
miR17-5p	Plasma	High	Turkish [19]	Tumor suppressor
	Serum	High	Canadian [18]	Inhibits cell migration [54]
miR-483-5p	Plasma	High	Turkish [19]	Oncogene
	Serum	High	Chinese [108]	Targets FIS1 in TSCC
		High	Canadian [18]	[110]
miR-26a	Plasma	High	Danish [25]	Tumor suppressor
		No change	Chinese [25]	Inhibits cell proliferation and cell cycle progression and induces apoptosis
	Serum	High	Canadian [18]	
miR-486-5p	Serum	High	Canadian [18]	Tumor suppressor
	Plasma	Low	Danish [25]	Inhibits cell proliferation and migration and induces apoptosis in ESCC [111]
		Low	Chinese [25]	

salivary miRNA screening emerges as a valuable diagnostic method for detection of human cancers, especially for those of the salivary glands and of oral mucosa.

Several miRNAs have been reported to show different levels in the saliva of OSCC patients compared to healthy controls (Table 2). MiR-136, miR-147, miR-220a, miR-323-5p, miR-503, miR-632, miR-646, miR-668, miR-877 and miR-1250 have been reported to show lower levels in the "saliva" of an American cohort of OSCC patients compared to that of healthy controls [42]. Unstimulated WMF was collected from patients and immediately processed followed by miRNA expression analysis using multiplexed NanoString nCounter miRNA expression assay. Further validations were done using quantitative real-time PCR [42]. Another independent study reported miR-125a and miR-200a to show significantly reduced levels in the WMF of OSCC patients [43]. Unlike Momen et al., they isolated RNA from the supernatant sample using mirVana™ miRNA Isolation Kit and detected the differentially expressed miRNAs using four-plex RT-preamp-qPCR. The NanoString nCounter miRNA expression assay is reported to be sensitive and the results are reproducible, however, to some extent, these depend on the miRNA isolation process. Hence, the different sampling procedure used by Park et al. (2009) could be a reason for different results among these studies. The disease state as well as different habits of the patients can also be the cause of these contradictory results. Two miRNAs, miR-139-5p [44] and miR-145 [45] are reported to show lower levels in the supernatant WMF of Turkish and Japanese populations respectively. A study in a Taiwanese cohort reported miR-145 and additional 7 miRNAs to be differentially expressed in OSCC patients compared to healthy controls. These differing reports might be interpreted as a population specific miRNA signature in OSCC patients, but different types of oral habits as well as different environmental conditions may also be the cause of these differences. Most importantly, the method of sample collection and storage plays a crucial role. Isolation of miRNAs from WMF supernatant, pellet or WMF itself can yield different miRNA expression profiles [46]. Since WMF consists of cellular debris and degraded RNAs, supernatant is probably a better sample for assaying cell free nucleic acids. Moreover, exosome enrichment followed by miRNA isolation would further reduce the variability in expression profiles among samples due to the removal of degraded RNAs.

Lack of well-characterized or matched clinical groups and lack of suitable endogenous controls for extracellular miRNA detection in WMF, and the need for normalization are among the major restrictions in applying salivary-based miRNA for biomarker discovery. Therefore, a larger scientific initiative involving large number of malignant cases from various populations needs to be performed for identifying appropriate salivary miRNA biomarkers to elucidate population specific, habit specific and stage of the disease specific miRNA signatures in OSCC patients.

4. Similar and dissimilar expression profiles of miRNAs in the saliva/WMF and serum/plasma of OSCC patients

Several miRNAs are reported to show similar concentrations in different body fluids of OSCC patients. For example, miR-24, miR-146a, miR-184, miR-31, miR-451 and miR-26a have been reported to be mostly uniformly expressed both in the saliva/WMF and serum or plasma of OSCC patients compared to healthy controls (Table 3). The presence of these miRNAs in different body fluids as well as among different populations suggests that they can be used as biomarkers for oral cancer detection irrespective of their oral habits or environmental factors. Moreover, several miRNAs are also reported to be associated with cancer recurrence. The expression levels of these miRNAs pre- and post-surgery can thus be important biomarkers for cancer recurrence [25].

On the contrary, certain miRNAs showed biphasic expression levels in different studies belonging to different population groups (Table 4). For example, miR-21 showed elevated levels in the saliva [47], serum

Table 2
miRNAs reported to show different levels in Saliva/whole mouth fluid (WMF) and their functional significance.

miRNA	Sample	miRNA level in OSCC vs. Normal	Demography	Functional role
miR-136	Unstimulated whole saliva	Low	American [42]	–
miR-147	Unstimulated whole saliva	Low	American [42]	–
miR-1250	Unstimulated whole saliva	Low	American [42]	–
miR-632	Unstimulated whole saliva	Low	American [42]	–
miR-646	Unstimulated whole saliva	Low	American [42]	–
miR-668	Unstimulated whole saliva	Low	American [42]	–
miR-877	Unstimulated whole saliva	Low	American [42]	–
miR-503	Unstimulated whole saliva	Low	American [42]	–
miR-220a	Unstimulated whole saliva	Low	American [42]	–
miR-323-5p	Unstimulated whole saliva	Low	American [42]	–
miR-200a	Saliva supernatant	Low	American [43]	Tumor suppressor Inhibits cell malignant transformation and tumor initiation
miR-145	WMF	Low	Japanese [45,47]	Tumor suppressor
		High	Taiwanese [46]	Inhibits OSCC cell growth targeting c-Myc and Cdk6 Targets FSCN1 in ESCC [112,113]
miR-10b-3p	WMF	High	Taiwanese [46]	Oncogene Targets PPARA and KLF4 [114]
miR-708	WMF	High	Taiwanese [46]	–
miR-139-5p	Saliva supernatant	Low	Turkish [44]	Tumor suppressor Inhibits cell proliferation in OSCC targeting TPD52
miR-125a	Saliva supernatant	Low	American [43]	Tumor suppressor Targets oncogenic proteins ERBB2 and ERBB3, Estrogen-related Receptor α [115,116]
miR-30e	Unstimulated whole saliva	High	Taiwanese [46]	–
miR-99b	Unstimulated whole saliva	Low	Taiwanese [46]	Tumor suppressor Inhibits cell proliferation targeting GSK3β in OSCC [92]
miR-660	Unstimulated whole saliva	High	Taiwanese [46]	–
miR-181b/c	Unstimulated whole saliva	Low	Taiwanese [46]	Tumor suppressor Targets p27kip1 and Bcl-2 Enhanced cell migration and invasion [66]
miR-197	Unstimulated whole saliva	Low	Taiwanese [46]	–
miR-9	Unstimulated whole saliva	Low	Taiwanese [46]	Tumor suppressor Targets CXC chemokine receptor 4 and NFκβ [84]
miR-24	Saliva supernatant	High	American [42]	Oncogene Facilitates growth of OSCC cells by targeting p57
miR-146a	Saliva supernatant	High	American [43]	Oncogene Increases tumorigenesis and metastasis in OSCC by targeting IRAK1, TRAF6 and NUMB genes [55]
miR-21	WMF	High	Arabs [47]	Oncogene
	Unstimulated whole saliva	High	Taiwanese [46]	Induces tumor cell invasion and proliferation by targeting PTEN and PCD4 [49]
miR-184	WMF	High	Arabs [47]	Oncogene Antiapoptosis and proliferation of TSCC, targeting SF1 [103]
miR-31	WMF	High	Taiwanese [105]	Oncogene miR-31 promotes OSCC by enhancing the migration and invasiveness of OSCC cells targeting ACOX1 [106,117]
miR-27b	Saliva supernatant	High	American [42]	Tumor suppressor Inhibits cancer cell migration and invasion targeting MET [107]
miR-148a	Saliva supernatant	Low	American [42]	Tumor suppressor Inhibits cancer cell migration and invasion targeting Wnt10b
miR-451	WMF	High	[118]	Tumor suppressor
miR-26a	WMF	High	Taiwanese [46]	Tumor suppressor Inhibits cell proliferation and cell cycle progression and induces apoptosis

[48], blood [49] and plasma [25,50] of OSCC patients from Japan, Netherlands, Germany and India compared to normal controls, but did not show any significant difference among OSCC patients of Taiwanese population [25]. In another study with a Turkish population, miR-21 is reported to show lower levels in the plasma of patients having benign salivary tumors [51]. This may be interpreted as the association of miR-21 specifically with malignant neoplasms. Similarly, miR-27b is reported to be elevated in the WMF of an American group of OSCC patients [42], but is found to be reduced in the plasma of OSCC patients from Taiwan [52]. MiR-148a also showed a contradictory expression levels in the plasma of two different population cohorts [25], while it showed reduced levels in the WMF of OSCC patients [42]. However, with this limited number of studies, it is difficult to ascertain their uniform or biphasic regulation across different body fluids. These miRNAs need to be validated among OSCC patients across different populations world-wide. Uniformity in sample collection, preparation and determination of expression profiles need to be standardized and reported in such biomarker studies to determine their reproducibility in other populations.

5. MiRNAs as prognostic biomarkers in oral cancer

The expression patterns of certain miRNAs have shown positive correlation with clinical stage, lymph node metastasis and patient survival, indicating that these miRNAs can act as prognostic predictors in OSCC. Higher expression levels of miR-372 is shown to induce nodal metastasis and poor prognosis of oral carcinoma [53], while miR-134 showed association with number of metastases in HNSCC [54]. MiR-146a is reported to show increased metastasis by down-regulating the expression of IRAK1, TRAF6 and NUMB [55]. This, however, is contradicted by another study where overexpression of miR-146a was shown to inhibit invasion, tumorigenicity, and metastasis in OSCC cell

Table 3
miRNAs (common) reported to show different levels in plasma/serum and WMF/Saliva.

miRNA	Sample	miRNA level in OSCC vs. Normal	Demography
miR-24	Plasma	High	Taiwanese [102]
	Saliva supernatant	High	American [42]
miR-146a	Plasma	High	Taiwanese [55]
	Saliva supernatant	High	American [43]
miR-184	Plasma	High	Chinese [98]
	Serum	High	Chinese [104]
	WMF	High	Arabs [47]
miR-31	WMF	High	Taiwanese [105]
	Plasma	High	Taiwanese [105]
		No change	Brazilian [97]
miR-451	Serum	High	Canadian [18]
	WMF	High	Chinese [118]
miR-26a	Plasma	High	Danish [25]
		No change	Chinese [25]
	Serum	High	Canadian [18]
	WMF	High	Chinese [46]

lines by targeting SOX2 mRNA [56]. Since, miR-146a is reported to show a biphasic expression, hence, deregulation of the downstream mRNAs needs to be analysed to comment on its prognostic and diagnostic potential in OSCC. Low expression of miR-150 in Esophageal Squamous Cell Carcinoma (ESCC) showed association with malignancy, such as tumor depth, lymph node metastasis, lymphatic invasion, venous invasion and poor prognosis [57]. MiR-338 is also a tumor suppressor miRNA in OSCC. It is reported to inhibit metastasis in hepatocellular carcinoma, lung cancer and gastric cancer as well [58–60]. Considering these observations, it could be proposed that an increased expression of miR-372 and miR-134, and a decreased expression of miR-150-5p and miR-338 might be a potential complex for detecting, and possibly predicting the risk of, metastasis in oral carcinoma (Fig. 1).

A higher expression level of miR-21 in Tongue Squamous Cell Carcinoma (TSCC) is correlated with advanced clinical stage, poor differentiation and lymph node metastasis suggesting its potential to be used as a prognostic marker for TSCC patient survival [61]. MiR-31, miR-17, miR-125b, miR-155, miR-181, miR-205, and miR-let7d were also found to be associated with lymph node metastasis and poor patient survival [39,62–67] and thus, could be used to detect the metastatic potential of cancer. Since a single miRNA targets multiple mRNAs, cancer prognosis and risk of metastasis might be correlated with both the miRNAs and their target mRNAs that are enriched in a particular tissue type. Cells enriched with oncogenic mRNAs would result in decreased tumor progression due to miRNA mediated post transcriptional repression of their target oncogenic mRNAs, while cells abundantly expressing tumor suppressor mRNAs would result in accelerated progression by miRNA mediated repression of their target tumor suppressor mRNAs. The expressions of target mRNAs also depend on the tissue types, clinical stages of the cancer, environmental risk factors and habits of the patients.

6. Therapeutic role of circulating miRNAs in oral cancer

The ability to manipulate miRNA expression and function by local and systemic delivery of miRNA inhibitors or miRNA mimics has recently gained immense interest as a novel therapeutic approach [68,69]. The advantage of miRNA based cancer therapy lies in the ability of miRNAs to concurrently target multiple effectors of pathways involved in cell proliferation, differentiation, and survival. However, the major challenges are in vivo delivery of these polyanionic oligonucleotides. Naked miRNAs cannot pass through hydrophobic cell membranes and are also prone to be degraded by RNase. Chemically modified miRNA-targeting antisense oligonucleotides and nanoparticle based delivery approaches have shown considerable promise in improving both bioavailability and stability of miRNAs. However, efficient targeting to specific areas of the body still remains challenging [70].

Resistance to chemotherapy radiotherapy are major challenges in the management of OSCC patients. Recent studies have linked resistance to chemo- and radiotherapy in OSCC to altered miRNA expression (Fig. 1). MiR-375, a tumor suppressor miRNA, is reported to be lower than normal in the plasma of OSCC patients [25,71]. It has been shown that the miR-375 inhibits growth and enhances radiosensitivity of OSCC by targeting IGF1R [71]. A decreased expression of miR-375 might be associated with resistance to radiotherapy and hence, overexpressing miR-375 by miRNA mimics might result in increased sensitivity and responsiveness to radiotherapy in OSCC patients. Another tumor suppressor, miRNA Let-7d, is found to be lower in the serum of OSCC patients [18]. Reduced expression of this miRNA showed increase in chemoresistance in OSCC patients [72]. Hence, targeted delivery of Let-7d mimics is expected to result in increased chemosensitivity and responsiveness of patients towards chemotherapy. A decreased expression of miR-200b is reported to be associated with chemotherapeutic resistance and poor prognosis of TSCC patients [73], whereas overexpression of miR-196a is associated with resistance to radiotherapy in HNSCC [74]. MiR-21 is an oncogenic miRNA that is associated with chemosensitivity of several human cancer cell lines to anticancer agents. For example, miR-21 modulates chemosensitivity of TSCC cells to cisplatin by targeting PDCD4 [75], while inhibition of miR-21 by anti-miRs or siRNA is reported to induce sensitivity of TSCC cells to cisplatin [75]. In a study by Shiiba et al., transfection of OSCC

Table 4
miRNAs reported to show differential profiles in serum/plasma and WMF/Saliva and possible reasons for their disconcordant levels.

miRNA	Sample	miRNA level in OSCC vs. Normal	Demography	Reasons for disconcordance
miR-21	Plasma	High	Japanese [50]	• The difference in miR-21 expression in the different studies performed on different population cohorts can be suggested as a population specific deregulation of miRNA signature in OSCC.
		No change	Chinese [25]	
		High	Danish [25]	• The expression of miR-21 was found to be down-regulated in case of benign salivary tumors in a Turkish cohort suggesting the association of miR-21 specific to malignant tumors.
		Low	Turkish [51]	
	Serum	High	Indian [48]	
	Blood	High	German [49]	
	WMF	High	Arabs [47]	
miR-27b	Saliva supernatant	High	American [42]	• Difference in sample type and the difference in the ethnicity of the population groups can be attributed to the difference in expression of miR-27b
	Plasma	Low	Taiwanese [52]	
miR-148a	Saliva supernatant	Low	American [42]	• miR-148 was studied in the plasma of a Danish cohort and Chinese cohort by the same group. Since, the collection procedure and handling was same, thus, the difference in expression of miR-148a might be attributed to the difference in population.
	Plasma	High	Danish [25]	
		No change	Chinese [25]	• Down-regulation in of miR-148a in the saliva of an American population might be due the difference in sample type or ethnicity.

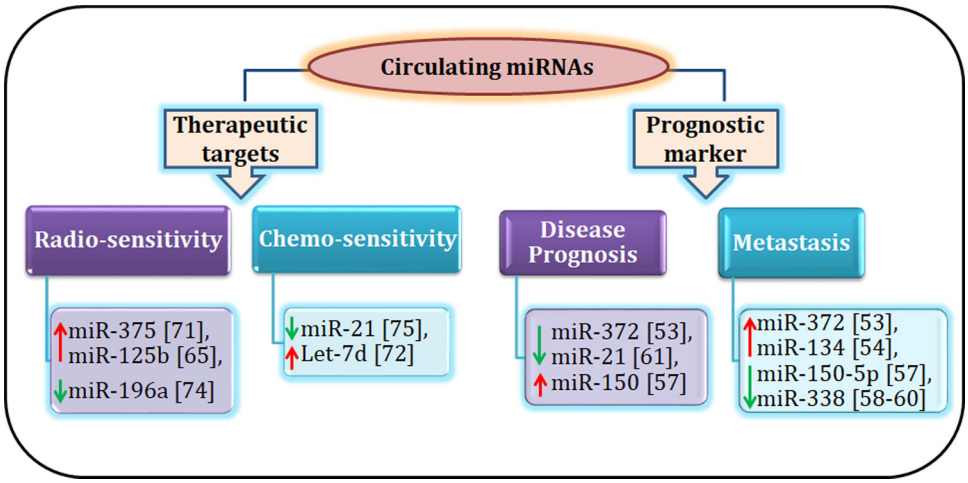

Fig. 1. Summarized representation of circulating miRNAs reported as therapeutic and prognostic markers in OSCC. The red up and green down arrows represent the high and low levels of miRNAs in the body fluids of OSCC patients with respect to disease prognosis or response to therapy (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

cell lines with miR-125b resulted in a decreased proliferation rate and enhanced radiosensitivity [65]. Hence, *in-vivo* delivery of this miRNA might result in enhanced response to treatment. Reduced expression of miR-100, miR-130a and miR-197 and an elevated expression of miR-181b, miR-181d, miR-101 and miR-195 are also reported to be correlated with multiple drug resistance in HNSCC [76]. Despite the promising therapeutic roles of miRNAs in cancer, further studies and elaborate research are required for the development of an effective *in-vivo* delivery system for optimal uptake and targeted delivery of miRNAs.

7. Conclusion

In oral cancer, the lesion is usually visible and easily accessible for procuring biopsy, therefore, liquid biopsy may sound redundant in oral cancer diagnostics. But, biopsy is an invasive procedure and involves physical pain to the patients. Hence, liquid biopsy plays a pivotal role in non-invasive detection of cancer at early stages of the disease.

Several miRNAs have been reported to be deregulated in the body fluids of OSCC patients compared to healthy controls suggesting that these miRNAs can be used as non-invasive biomarkers for oral cancer detection. Several population cohorts have shown different miRNA signatures, perhaps signifying population specific deregulation of miRNAs owing to differences in lifestyle habits, an inherited genetic component and other environmental factors. Circulating miRNAs might serve as biomarkers for risk of oral cancer development, for prognosis and response to treatment. Despite recent advances in the field of liquid biopsy, technical challenges remain. Proper sample handling, isolation and normalization of miRNAs are important parameters for evaluating their expression. Exosomal purification and isolation of RNA from body fluids and normalization with an exogenous control are important to reduce variation due to RNA degradation. A therapeutic role for miRNAs requires further work on a suitable *in-vivo* delivery device or mechanism [77,78].

Funding

This work was supported by the CSIR extramural (Grant no 27(0306)/14//EMR-II) to RC, JR and KC and ISI intramural funding to RC.

Conflict of interest

The authors declare no relevant conflicts of interest.

Acknowledgments

SM is thankful to CSIR for providing the CSIR-NET fellowship.

References

[1] B. Gupta, N.W. Johnson, N. Kumar, Global epidemiology of head and neck cancers: a continuing challenge, Oncology 91 (1) (2016) 13–23.
[2] F. Bray, et al., Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J. Clin. 68 (6) (2018) 394–424.
[3] L.A. Torre, et al., Global cancer statistics, 2012, CA Cancer J. Clin. 65 (2) (2015) 87–108.
[4] B. Basu, et al., Genome-wide DNA methylation profile identified a unique set of differentially methylated immune genes in oral squamous cell carcinoma patients in India, Clin. Epigenet. 9 (2017) 13.
[5] J.P. Shah, N.W. Johnson, J.P. Shah, N.W. Johnson (Eds.), Oral and Oropharyngeal Cancer, 2nd ed., CRC Press, 2018, p. 553.
[6] E. Crowley, et al., Liquid biopsy: monitoring cancer-genetics in the blood, Nat. Rev. Clin. Oncol. 10 (8) (2013) 472–484.
[7] A. Diamantis, E. Magiorkinis, H. Koutselini, Fine-needle aspiration (FNA) biopsy: historical aspects, Folia Histochem. Cytobiol. 47 (2) (2009) 191–197.
[8] H. Schwarzenbach, D.S. Hoon, K. Pantel, Cell-free nucleic acids as biomarkers in cancer patients, Nat. Rev. Cancer 11 (6) (2011) 426–437.
[9] C. Roth, et al., Screening for circulating nucleic acids and caspase activity in the peripheral blood as potential diagnostic tools in lung cancer, Mol. Oncol. 5 (3) (2011) 281–291.
[10] Ginkel, Cell-free nucleic acids in body fluids as biomarkers for the prediction and early detection of recurrent head and neck cancer: a systematic review of the literature, Oral Oncol. 75 (2017) 8–15.
[11] H. Mansour, Cell-free nucleic acids as noninvasive biomarkers for colorectal cancer detection, Front. Genet. 5 (2014) 182.
[12] V. Vasioukhin, et al., Point mutations of the N-ras gene in the blood plasma DNA of patients with myelodysplastic syndrome or acute myelogenous leukaemia, Br. J. Haematol. 86 (4) (1994) 774–779.
[13] C. Thery, M. Ostrowski, E. Segura, Membrane vesicles as conveyors of immune responses, Nat. Rev. Immunol. 9 (8) (2009) 581–593.
[14] N. Kosaka, H. Iguchi, T. Ochiya, Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis, Cancer Sci. 101 (10) (2010) 2087–2092.
[15] J.A. Weber, et al., The microRNA spectrum in 12 body fluids, Clin. Chem. 56 (11) (2010) 1733–1741.
[16] M.A. Cortez, et al., MicroRNAs in body fluids—the mix of hormones and biomarkers, Nat. Rev. Clin. Oncol. 8 (8) (2011) 467–477.
[17] E. Bell, M.A. Taylor, Functional roles for exosomal microRNAs in the tumour microenvironment, Comput. Struct. Biotechnol. J. 15 (2017) 8–13.
[18] S.A. Maclellan, et al., Differential expression of miRNAs in the serum of patients with high-risk oral lesions, Cancer Med. 1 (2) (2012) 268–274.
[19] L. Ayaz, et al., Differential expression of microRNAs in plasma of patients with laryngeal squamous cell carcinoma: potential early-detection markers for laryngeal squamous cell carcinoma, J. Cancer Res. Clin. Oncol. 139 (9) (2013) 1499–1506.
[20] T. Blondal, et al., Assessing sample and miRNA profile quality in serum and plasma or other biofluids, Methods 59 (1) (2013) S1–S6.
[21] K. Wang, et al., Export of microRNAs and microRNA-protective protein by mammalian cells, Nucleic Acids Res. 38 (20) (2010) 7248–7259.
[22] C. Chakraborty, S. Das, Profiling cell-free and circulating miRNA: a clinical diagnostic tool for different cancers, Tumour Biol. 37 (5) (2016) 5705–5714.
[23] J.D. Arroyo, et al., Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma, Proc. Natl. Acad. Sci. U. S. A. 108 (12) (2011) 5003–5008.
[24] A. Turchinovich, et al., Characterization of extracellular circulating microRNA, Nucleic Acids Res. 39 (16) (2011) 7223–7233.
[25] Y. Yan, et al., Circulating miRNAs as biomarkers for oral squamous cell carcinoma

recurrence in operated patients, Oncotarget 8 (5) (2017) 8206–8214.
[26] R. Shah, et al., Discordant expression of circulating microRNA from cellular and extracellular sources, PLoS One 11 (4) (2016) e0153691.
[27] K. Wang, et al., Comparing the microRNA spectrum between serum and plasma, PLoS One 7 (7) (2012) e41561.
[28] H. Ple, et al., The repertoire and features of human platelet microRNAs, PLoS One 7 (12) (2012) e50746.
[29] H.H. Cheng, et al., Plasma processing conditions substantially influence circulating microRNA biomarker levels, PLoS One 8 (6) (2013) e64795.
[30] M.B. Kirschner, et al., The impact of hemolysis on cell-free microRNA biomarkers, Front. Genet. 4 (2013) 94.
[31] V. Koberle, et al., Differential stability of cell-free circulating microRNAs: implications for their utilization as biomarkers, PLoS One 8 (9) (2013) e75184.
[32] D.J. Kim, et al., Plasma components affect accuracy of circulating cancer-related microRNA quantitation, J. Mol. Diagn. 14 (1) (2012) 71–80.
[33] J.A. Makarova, et al., Circulating microRNAs, Biochemistry (Mosc.) 80 (9) (2015) 1117–1126.
[34] K.E.A. Max, et al., Human plasma and serum extracellular small RNA reference profiles and their clinical utility, Proc. Natl. Acad. Sci. U. S. A. 115 (23) (2018) E5334–E5343.
[35] S.P. Humphrey, R.T. Williamson, A review of saliva: normal composition, flow, and function, J. Prosthet. Dent. 85 (2) (2001) 162–169.
[36] T. Shpitzer, et al., Salivary analysis of oral cancer biomarkers, Br. J. Cancer 101 (7) (2009) 1194–1198.
[37] N.J. Bonne, D.T. Wong, Salivary biomarker development using genomic, proteomic and metabolomic approaches, Genome Med. 4 (10) (2012) 82.
[38] Y. Li, et al., Salivary transcriptome diagnostics for oral cancer detection, Clin. Cancer Res. 10 (24) (2004) 8442–8450.
[39] C.J. Liu, et al., Exploiting salivary miR-31 as a clinical biomarker of oral squamous cell carcinoma, Head Neck 34 (2) (2012) 219–224.
[40] J.M. Yoshizawa, D.T. Wong, Salivary microRNAs and oral cancer detection, Methods Mol. Biol. 936 (2013) 313–324.
[41] C.Z. Chen, MicroRNAs as oncogenes and tumor suppressors, N. Engl. J. Med. 353 (17) (2005) 1768–1771.
[42] F. Momen-Heravi, et al., Genomewide study of salivary microRNAs for detection of oral cancer, J. Dent. Res. 93 (7 Suppl) (2014) 86S–93S.
[43] N.J. Park, et al., Salivary microRNA: discovery, characterization, and clinical utility for oral cancer detection, Clin. Cancer Res. 15 (17) (2009) 5473–5477.
[44] M.B. Duz, et al., Identification of miR-139-5p as a saliva biomarker for tongue squamous cell carcinoma: a pilot study, Cell. Oncol. (Dordr.) 39 (2) (2016) 187–193.
[45] I. Igaz, P. Igaz, Diagnostic relevance of microRNAs in other body fluids including urine, feces, and saliva, EXS 106 (2015) 245–252.
[46] Y. Yang, et al., Progress risk assessment of oral premalignant lesions with saliva miRNA analysis, BMC Cancer 13 (2013) 129.
[47] F. Zahran, et al., Salivary microRNAs in oral cancer, Oral Dis. 21 (6) (2015) 739–747.
[48] P. Singh, et al., Circulating microRNA-21 expression as a novel serum biomarker for oral sub-mucous fibrosis and oral squamous cell carcinoma, Asian Pac. J. Cancer Prev. 19 (4) (2018) 1053–1057.
[49] P.P. Reis, et al., Programmed cell death 4 loss increases tumor cell invasion and is regulated by miR-21 in oral squamous cell carcinoma, Mol. Cancer 9 (2010) 238.
[50] H. Tachibana, et al., Circulating miR-223 in oral cancer: its potential as a novel diagnostic biomarker and therapeutic target, PLoS One 11 (7) (2016) e0159693.
[51] O. Cinpolat, et al., Comparison of microRNA profiles between benign and malignant salivary gland tumors in tissue, blood and saliva samples: a prospective, case-control study, Braz. J. Otorhinolaryngol. 83 (3) (2017) 276–284.
[52] W.Y. Lo, et al., miR-27b-regulated TCTP as a novel plasma biomarker for oral cancer: from quantitative proteomics to post-transcriptional study, J. Proteom. 77 (2012) 154–166.
[53] H.F. Tu, et al., Upregulation of miR-372 and -373 associates with lymph node metastasis and poor prognosis of oral carcinomas, Laryngoscope 125 (11) (2015) E365–70.
[54] C.J. Liu, et al., miR-134 induces oncogenicity and metastasis in head and neck carcinoma through targeting WWOX gene, Int. J. Cancer 134 (4) (2014) 811–821.
[55] P.S. Hung, et al., miR-146a enhances the oncogenicity of oral carcinoma by concomitant targeting of the IRAK1, TRAF6 and NUMB genes, PLoS One 8 (11) (2013) e79926.
[56] Z. Shi, et al., Decrease of miR-146a is associated with the aggressiveness of human oral squamous cell carcinoma, Arch. Oral Biol. 60 (9) (2015) 1416–1427.
[57] T. Yokobori, et al., MiR-150 is associated with poor prognosis in esophageal squamous cell carcinoma via targeting the EMT inducer ZEB1, Cancer Sci. 104 (1) (2013) 48–54.
[58] J.S. Chen, et al., miR-338-3p inhibits epithelial-mesenchymal transition and metastasis in hepatocellular carcinoma cells, Oncotarget 8 (42) (2017) 71418–71429.
[59] X. Chen, L. Wei, S. Zhao, miR-338 inhibits the metastasis of lung cancer by targeting integrin beta3, Oncol. Rep. 36 (3) (2016) 1467–1474.
[60] Y. Peng, et al., MicroRNA-338 inhibits growth, invasion and metastasis of gastric cancer by targeting NRP1 expression, PLoS One 9 (4) (2014) e94422.
[61] J. Li, et al., MiR-21 indicates poor prognosis in tongue squamous cell carcinomas as an apoptosis inhibitor, Clin. Cancer Res. 15 (12) (2009) 3998–4008.
[62] L.J. Shi, et al., MicroRNA-155 in oral squamous cell carcinoma: overexpression, localization, and prognostic potential, Head Neck 37 (7) (2015) 970–976.
[63] W.C. Huang, et al., miRNA-491-5p and GIT1 serve as modulators and biomarkers for oral squamous cell carcinoma invasion and metastasis, Cancer Res. 74 (3) (2014) 751–764.
[64] C.C. Chang, et al., MicroRNA-17/20a functions to inhibit cell migration and can be used a prognostic marker in oral squamous cell carcinoma, Oral Oncol. 49 (9) (2013) 923–931.
[65] M. Shiiba, et al., MicroRNA-125b regulates proliferation and radioresistance of oral squamous cell carcinoma, Br. J. Cancer 108 (9) (2013) 1817–1821.
[66] C.C. Yang, et al., miR-181 as a putative biomarker for lymph-node metastasis of oral squamous cell carcinoma, J. Oral Pathol. Med. 40 (5) (2011) 397–404.
[67] G. Childs, et al., Low-level expression of microRNAs let-7d and miR-205 are prognostic markers of head and neck squamous cell carcinoma, Am. J. Pathol. 174 (3) (2009) 736–745.
[68] R. Garzon, G. Marcucci, C.M. Croce, Targeting microRNAs in cancer: rationale, strategies and challenges, Nat. Rev. Drug Discov. 9 (10) (2010) 775–789.
[69] M.S. Ebert, J.R. Neilson, P.A. Sharp, MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells, Nat. Methods 4 (9) (2007) 721–726.
[70] V. Baumann, J. Winkler, miRNA-based therapies: strategies and delivery platforms for oligonucleotide and non-oligonucleotide agents, Future Med. Chem. 6 (17) (2014) 1967–1984.
[71] B. Zhang, et al., MicroRNA-375 inhibits growth and enhances radiosensitivity in oral squamous cell carcinoma by targeting insulin like growth factor 1 receptor, Cell. Physiol. Biochem. 42 (5) (2017) 2105–2117.
[72] C.J. Chang, et al., Let-7d functions as novel regulator of epithelial-mesenchymal transition and chemoresistant property in oral cancer, Oncol. Rep. 26 (4) (2011) 1003–1010.
[73] L. Sun, et al., MiR-200b and miR-15b regulate chemotherapy-induced epithelial-mesenchymal transition in human tongue cancer cells by targeting BMI1, Oncogene 31 (4) (2012) 432–445.
[74] Y.E. Suh, et al., MicroRNA-196a promotes an oncogenic effect in head and neck cancer cells by suppressing annexin A1 and enhancing radioresistance, Int. J. Cancer 137 (5) (2015) 1021–1034.
[75] W. Ren, et al., MiR-21 modulates chemosensitivity of tongue squamous cell carcinoma cells to cisplatin by targeting PDCD4, Mol. Cell. Biochem. 390 (1–2) (2014) 253–262.
[76] Y. Dai, et al., MicroRNA expression profiles of head and neck squamous cell carcinoma with docetaxel-induced multidrug resistance, Head Neck 33 (6) (2011) 786–791.
[77] M.H. Shaikh, et al., Aurora kinases are a novel therapeutic target for HPV-positive head and neck cancers, Oral Oncol. 86 (2018) 105–112.
[78] M.H. Shaikh, et al., Can gene editing and silencing technologies play a role in the treatment of head and neck cancer? Oral Oncol. 68 (2017) 9–19.
[79] M. Manikandan, et al., Oral squamous cell carcinoma: microRNA expression profiling and integrative analyses for elucidation of tumourigenesis mechanism, Mol. Cancer 15 (2016) 28.
[80] D. Poel, et al., Evaluation of several methodological challenges in circulating miRNA qPCR studies in patients with head and neck cancer, Exp. Mol. Med. 50 (3) (2018) e454.
[81] C. Liu, et al., MiR-338 suppresses the growth and metastasis of OSCC cells by targeting NRP1, Mol. Cell. Biochem. 398 (1–2) (2015) 115–122.
[82] L. Lu, et al., MicroRNA-29a upregulates MMP2 in oral squamous cell carcinoma to promote cancer invasion and anti-apoptosis, Biomed. Pharmacother. 68 (1) (2014) 13–19.
[83] L. Sun, et al., Association of decreased expression of serum miR-9 with poor prognosis of oral squamous cell carcinoma patients, Med. Sci. Monit. 22 (2016) 289–294.
[84] T. Yu, et al., MicroRNA-9 inhibits the proliferation of oral squamous cell carcinoma cells by suppressing expression of CXCR4 via the Wnt/beta-catenin signaling pathway, Oncogene 33 (42) (2014) 5017–5027.
[85] N. Xie, et al., Decreased miR-320a promotes invasion and metastasis of tumor budding cells in tongue squamous cell carcinoma, Oncotarget 7 (40) (2016) 65744–65757.
[86] L. Jiang, et al., MicroRNA-7 targets IGF1R (insulin-like growth factor 1 receptor) in tongue squamous cell carcinoma cells, Biochem. J. 432 (1) (2010) 199–205.
[87] H.M. Jung, et al., Keratinization-associated miR-7 and miR-21 regulate tumor suppressor reversion-inducing cysteine-rich protein with kazal motifs (RECK) in oral cancer, J. Biol. Chem. 287 (35) (2012) 29261–29272.
[88] J.Y. Xu, et al., MiR-25-3p attenuates the proliferation of tongue squamous cell carcinoma cell line Tca8113, Asian Pac. J. Trop. Med. 6 (9) (2013) 743–747.
[89] T. Wang, et al., miR-195-5p suppresses the proliferation, migration, and invasion of oral squamous cell carcinoma by targeting TRIM14, Biomed Res. Int. 2017 (2017) 7378148.
[90] C.T. Dickman, et al., Selective extracellular vesicle exclusion of miR-142-3p by oral cancer cells promotes both internal and extracellular malignant phenotypes, Oncotarget 8 (9) (2017) 15252–15266.
[91] Y.C. Lu, et al., Combined determination of circulating miR-196a and miR-196b levels produces high sensitivity and specificity for early detection of oral cancer, Clin. Biochem. 48 (3) (2015) 115–121.
[92] K. He, et al., miRNA-99b-3p functions as a potential tumor suppressor by targeting glycogen synthase kinase-3beta in oral squamous cell carcinoma Tca-8113 cells, Int. J. Oncol. 47 (4) (2015) 1528–1536.
[93] P. Li, et al., MiR-194 functions as a tumor suppressor in laryngeal squamous cell carcinoma by targeting Wee1, J. Hematol. Oncol. 10 (1) (2017) 32.
[94] H. Chi, miR-194 regulated AGK and inhibited cell proliferation of oral squamous cell carcinoma by reducing PI3K-Akt-FoxO3a signaling, Biomed. Pharmacother. 71 (2015) 53–57.
[95] T.D. Kabir, et al., A miR-335/COX-2/PTEN axis regulates the secretory phenotype of senescent cancer-associated fibroblasts, Aging (Albany N. Y.) 8 (8) (2016) 1608–1635.

[96] Y. Hashiguchi, et al., Tumor-suppressive roles of DeltaNp63beta-miR-205 axis in epithelial-mesenchymal transition of oral squamous cell carcinoma via targeting ZEB1 and ZEB2, J. Cell. Physiol. 233 (10) (2017) 6565–6577.
[97] P. Severino, et al., Small RNAs in metastatic and non-metastatic oral squamous cell carcinoma, BMC Med. Genom. 8 (2015) 31.
[98] C.J. Liu, et al., Plasma miR-187* is a potential biomarker for oral carcinoma, Clin. Oral Invest. 21 (4) (2017) 1131–1138.
[99] G. Sun, et al., miR-200b-3p in plasma is a potential diagnostic biomarker in oral squamous cell carcinoma, Biomarkers (2017) 1–5.
[100] A.F. Christopher, M. Gupta, P. Bansal, Micronome revealed miR-19a/b as key regulator of SOCS3 during cancer related inflammation of oral squamous cell carcinoma, Gene 594 (1) (2016) 30–40.
[101] B.S. Li, et al., Plasma microRNAs, miR-223, miR-21 and miR-218, as novel potential biomarkers for gastric cancer detection, PLoS One 7 (7) (2012) e41629.
[102] S.C. Lin, et al., miR-24 up-regulation in oral carcinoma: positive association from clinical and in vitro analysis, Oral Oncol. 46 (3) (2010) 204–208.
[103] Z. Fang, et al., LncRNA UCA1 promotes proliferation and cisplatin resistance of oral squamous cell carcinoma by sunppressing miR-184 expression, Cancer Med. 6 (12) (2017) 2897–2908.
[104] J. Wittmann, H.M. Jack, Serum microRNAs as powerful cancer biomarkers, Biochim. Biophys. Acta 1806 (2) (2010) 200–207.
[105] C.J. Liu, et al., Increase of microRNA miR-31 level in plasma could be a potential marker of oral cancer, Oral Dis. 16 (4) (2010) 360–364.
[106] Y.H. Lai, et al., MiR-31-5p-ACOX1 axis enhances tumorigenic fitness in oral squamous cell carcinoma via the promigratory prostaglandin E2, Theranostics 8 (2) (2018) 486–504.
[107] I. Fukumoto, et al., The tumor-suppressive microRNA-23b/27b cluster regulates the MET oncogene in oral squamous cell carcinoma, Int. J. Oncol. 49 (3) (2016) 1119–1129.
[108] H. Xu, et al., Serum miR-483-5p: a novel diagnostic and prognostic biomarker for patients with oral squamous cell carcinoma, Tumour Biol. 37 (1) (2016) 447–453.
[109] J. Ries, et al., miR-186, miR-3651 and miR-494: potential biomarkers for oral squamous cell carcinoma extracted from whole blood, Oncol. Rep. 31 (3) (2014) 1429–1436.
[110] S. Fan, et al., miR-483-5p determines mitochondrial fission and cisplatin sensitivity in tongue squamous cell carcinoma by targeting FIS1, Cancer Lett. 362 (2) (2015) 183–191.
[111] Y. Yi, et al., Downregulated miR-486-5p acts as a tumor suppressor in esophageal squamous cell carcinoma, Exp. Ther. Med. 12 (5) (2016) 3411–3416.
[112] Y. Shao, et al., MiR-145 inhibits oral squamous cell carcinoma (OSCC) cell growth by targeting c-Myc and Cdk6, Cancer Cell Int. 13 (1) (2013) 51.
[113] M. Kano, et al., miR-145, miR-133a and miR-133b: tumor-suppressive miRNAs target FSCN1 in esophageal squamous cell carcinoma, Int. J. Cancer 127 (12) (2010) 2804–2814.
[114] O.F. Karatas, et al., MicroRNAs in human tongue squamous cell carcinoma: from pathogenesis to therapeutic implications, Oral Oncol. 67 (2017) 124–130.
[115] A. Tiwari, et al., MicroRNA-125a reduces proliferation and invasion of oral squamous cell carcinoma cells by targeting estrogen-related receptor alpha: implications for cancer therapeutics, J. Biol. Chem. 289 (46) (2014) 32276–32290.
[116] D.T. Wong, Salivary extracellular noncoding RNA: emerging biomarkers for molecular diagnostics, Clin. Ther. 37 (3) (2015) 540–551.
[117] K.W. Chang, et al., Passenger strand miRNA miR-31* regulates the phenotypes of oral cancer cells by targeting RhoA, Oral Oncol. 49 (1) (2013) 27–33.
[118] J. Du, L. Zhang, Analysis of salivary microRNA expression profiles and identification of novel biomarkers in esophageal cancer, Oncol. Lett. 14 (2) (2017) 1387–1394.