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Short Communication

Association of salivary miRNAs with onset and progression of oral potentially malignant disorders: Searching for noninvasive biomarkers

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Abstract *Background/purpose:* There is an urgent need for noninvasive biomarkers to diagnose oral potentially malignant disorders (OPMD). A wide range of over 20 miRNAs in saliva of OPMD patients have been investigated in different studies. Yet, which of the ones provide a better power of discrimination for the diagnosis of OPMD onset and progression are uncertain. *Materials and methods:* A total of 17 eligible studies including 426 cases of OPMD and 486 control subjects (352 normal mucosa and 134 oral squamous cell carcinoma) were summarized. *Results:* The bubble chart analysis showed that the most power salivary miRNA associated with OPMD onset was miR-21, followed by miR-31 and miR-142; the better power miRNAs associated with recurrence and malignant progression of OPMD were miR-31, miR-21, and miR-184. *Conclusion:* Salivary miRNAs, especially miR-21 and miR-31, were associated with onset and

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progression of OPMD, and could then serve as noninvasive biomarkers for screening OPMD and detecting malignant changes.

Introduction

Oral potentially malignant disorders (OPMD) carry a significantly increased risk for malignant transformation to oral squamous cell carcinoma (OSCC), which arises as a result of a multi-step carcinogenic process that correlates to the accumulation of epigenetic and genetic alterations.[1] Clinically, OPMD include a group of lesions such as oral leukoplakia (OLK), erythroplakia, oral lichen planus (OLP), and oral submucous fibrosis (OSF). Histologically, epithelial dysplasia is the pathological feature and malignant risk indicator of OPMD.[1] Nowadays, the gold standard for histopathological diagnosis of OPMD needs invasive detections such as incisional or excisional biopsy. Nevertheless, it is well-known that biopsy with histologic assessment is insufficient and may involve subjectivity.[1] Therefore, there is an urgent need for noninvasive biomarkers to screen the high-risk OPMD and improve the early detection of OSCC.

Increasing evidence indicates that aberrant microRNAs (miRNAs) in saliva sample of patients with OSCC represent a new paradigm in the development of noninvasive biomarkers for the clinical application of the disease.[2] Recent review studies have demonstrated that salivary miRNAs are a promising diagnostic tool with moderate accuracy for head and neck cancer diagnosis.[2] In the last years, over 20 kinds of miRNAs have been selected into the investigation of the diagnostic value for OPMD using saliva in different studies.[3—19] For instance, Tu et al.[19] recently reported decreased level of salivary miR-375 was involved in the multi-step oral carcinogenesis from normal mucosa, non-dysplasia, dysplasia, to OSCC development. Arguably, the combination of multiple salivary miRNAs may provide a better power of discrimination for the diagnosis of OPMD onset and progression to OSCC.

However, which of salivary miRNAs are the prominent biomarkers contributed for the process of OPMD are uncertain, partly due to lack of the systematic analysis focused on salivary miRNAs in OPMD patients. Therefore, we, to begin with, summarize these publications focused on this issue; and then identify a feasible proposal panel of prominent miRNAs by a visual bubble analysis method. This will provide the pertinent evidence for the investigators to choose rational miRNAs into the study design.

Materials and methods

In order to retrieve the relevant papers on salivary miRNAs in OPMD patients, literature search in PubMed, Web of Science, and Scopus databases was performed. We searched electronic databases without any restriction following the Cochrane collaboration and preferred reporting items for systematic reviews and meta-analyses guidelines. According to the search strategy described in Supplementary Table S1, we used medical subject term "OPMD", "miRNAs", "saliva", and their synonyms in the Title/Abstract. All the association studies about salivary miRNAs in OPMD patients published up to Jul 20, 2022 have been included in this study. Studies without sufficient data on OPMD research, even though there were OSCC and healthy control (HC) in the papers, were excluded. Two independent authors (X.S. and W.L.) screened the titles, abstracts, and full text of all publications, and subsequently analyzed the studies.

A descriptive analysis was performed on the parameters of included studies. The data on the number of patients and control and the number of the article with significant results was extracted to carried out a bubble chart analysis. A bubble chart used to visualize and interpret the power of salivary miRNAs in OPMD. The bubble chart for each miRNA shows the number of OPMD patients and healthy/OSCC controls and the number of the studies by the vertical/horizontal axis and bubble sizes. All the bubbles constitute a dashboard where the numbers of the subjects and studies in each theme bubble could be viewed by hovering over each data point. Excel Visual Basic for Applications (Microsoft 365, WA, USA) was used to programme a module to plot the bubble chart.

Results

Association of salivary miRNAs with OPMD onset

As presented in Supplementary Fig. S1, a total of 17 eligible studies containing 23 differentially expressed miRNAs were identified for detailed evaluation from literature databases (Table 1). There were 426 cases of OPMD and 486 control subjects (352 HC and 134 OSCC patients). Of these studies, 3 were follow-up studies, and the remaining 14 were comparative studies; 8 studies examined a significant miRNA, and the remaining 9 examined multiple miRNAs. As shown in Fig. 1A, the bubble chart analysis shows that the prominent salivary miRNAs associated with OPMD onset were miR-21, miR-31, and miR-142, which were measured by larger sample size of subjects. miR-21 upregulation was demonstrated by 5 studies including 187 cases of OPMD and 158 HC. miR-31 upregulation was confirmed by 3 studies including 85 cases and 75 HC. Besides, miR-27b downregulation was mainly associated with OLP onset, due to investigation by 3 studies including 39 cases of OLP and 39 HC.

Association of salivary miRNAs with OPMD progression

To assess the association of salivary miRNAs with disease progression of OPMD, we summarize the miRNAs were

Table 1 Summary of the studies on salivary miRNAs in oral potentially malignant disorders (OPMD).

Author, year	Country/Region	Study design	Sample size	Sample type	Significant miRNA examined
Liu et al., 2012[3]	China, Taiwan	Comparative	45 OSCC, 10 OLK, 24 HC	Saliva	miR-31↑
Yang et al., 2013[4]	China	Follow-up (3—5 years)	8 MT-OLK, 7 UT-OLK, 7 HC	Saliva	[a]miR-10b, -708 ↑; -145, -99b, -181c↓
Momen-Heravi et al., 2014[5]	USA	Comparative	9 OSCC, 8 OSCC in remission, 8 OLP, 9 HC	WUS	miR-27b↑; -29a, -146a, -223↓
Byun et al., 2015[6]	Korea	Comparative	14 OLP, 6 HC	WUS	miR-4484↑
Zahran et al., 2015[7]	Saudi Arabia	Comparative	20 OSCC, 20 dysplasia, 20 non-dysplasia, 20 HC	WUS	miR-21, -184↑; -145 ↓
Hung et al., 2016[8]	Taiwan	Follow-up (mean, 2.3 years)	20 OPMD, 24 HC	WUS	miR-21, -31↑
Shahidi et al., 2017[9]	Iran	Comparative	10 non-dysplastic OLP, 22 dysplastic OLP, 15 OSCC, 15 HC	WUS	miR-320a↓
Aghbari et al., 2018[10]	Egypt	Comparative	20 OLP, 20 HC	WUS	miR-27b, -137↓
Shah et al., 2018[11]	India	Comparative	30 OSCC, 10 OLK, 10 HC	Saliva	miR-34a ↓
Mehdipour et al., 2018[12]	Iran	Comparative	15 OSCC, 15 dysplastic OLP, 15 non-dysplastic OLP, 15 HC	WUS	miR-21, -31↑; -125a ↓
Stasio et al., 2019[13]	Italy	Comparative	5 OLP, 5 HC	WUS	miR-27b↓
Du et al., 2020[14]	China	Comparative	14 OLP, 14 HC	Saliva	miR-26a, -26b↓
Ge et al., 2020[15]	China	Comparative	14 OLP, 14 HC	Saliva	miR-27a, -27b↓
Prasad et al., 2020[16]	India	Comparative	61 OSF; 63 HC	WUS	miR-21↑
Uma et al., 2020[17]	India	Comparative	36 OPMD, 36 HC	WUS	miR-21, -31↑
Meng et al., 2021[18]	China	Comparative	56 OLP, 44 HC	Saliva	miR-142 ↑
Tu et al., 2022[19]	China, Taiwan	Follow-up (6.8 years)	41 OPMD, 26 HC	Saliva	miR-375 ↓

Abbr.: OSCC, oral squamous cell carcinoma; HC, healthy control; OLK, oral leukoplakia; OLP, oral lichen planus; OSF, oral submucous fibrosis; MT, malignant transformed; UT, untransformed; WUS, whole unstimulated saliva; ↑, upregulated; ↓, downregulated.

[a] These miRNAs were confirmed by both miRNA-microarray and RT-qPCR.

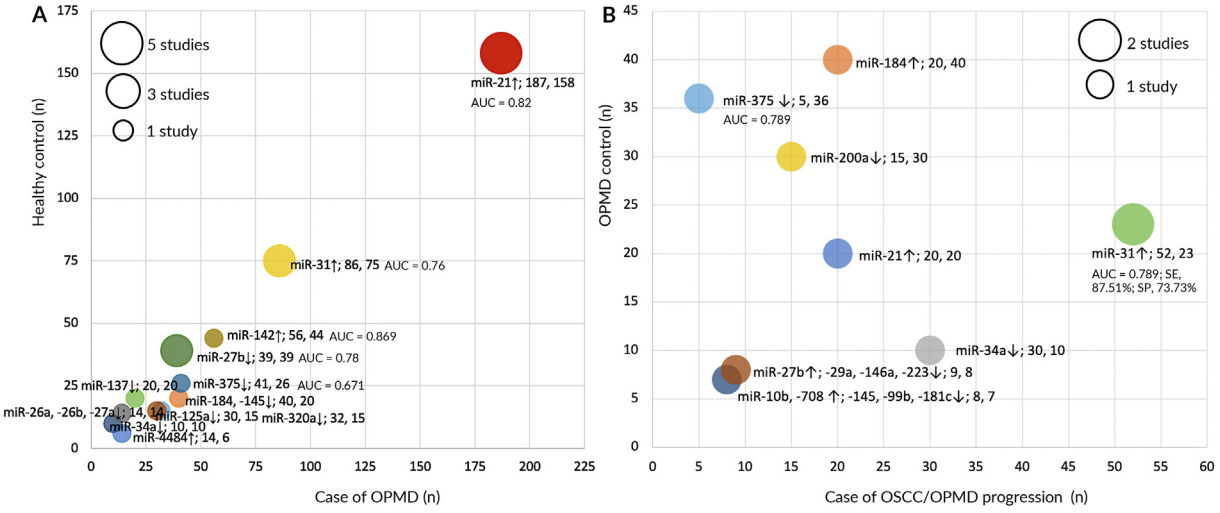

Figure 1 Bubble chart graphically represents the weighted value of the studies on salivary miRNAs in oral potentially malignant disorders (OPMD). (A) The number in the bubbles represents the sample size of OPMD vs. healthy control, respectively. (B) The number in the bubbles represents the sample size of OSCC or OPMD progression to OSCC vs. OPMD, respectively. The bubble diameter is proportionate to the number of studies included. The available diagnostic data is also presented in the dashboard. For instance, the AUC value of miR-31 was 0.789 with the sensitivity/specificity being 87.51%/73.73% for diagnosis OPMD progression. AUC, area under the curve; SE, sensitivity; SP, specificity; ↑, upregulated; ↓, downregulated.

differentially expressed between OSCC and OPMD and could predict the recurrence and malignant transformation of OPMD. As shown in Fig. 1B, the bubble chart analysis shows that salivary miRNAs associated with disease progression of OPMD scatter in the bubble dashboard. Salivary miR-31 upregulation in the disease progression was confirmed by 2 studies. Salivary miR-21 upregulation in 20 OSCC compared with 20 OPMD and miR-184 upregulation in 20 OSCC compared with 40 OPMD were reported. We observed that miR-21, miR-31, miR-184, and miR-375 participated in the disease process from normal mucosa, non-dysplasia, dysplasia, to OSCC development (see Fig. 1 and Supplementary Fig. S2). And, miR-27b, miR-137, and miR-142 mainly involve in the pathogenesis and diseases activity of OLP. Besides, miR-21, miR-31, and miR-375 are demonstrated to be of the predictive value for malignant transformation of OPMD by follow-up studies, although both sample sizes are small.

Discussion

Aberrant miRNAs have a crucial role in epigenetic regulation of various cellular biological processes of OSCC.[20] The clinical significances of critical miRNAs, i.e., miR-21, miR-31, and miR-375, in OSCC have been comprehensively reviewed and discussed elsewhere.[21,22] miRNA dysregulation is a critical step in the early phase of oral carcinogenesis, allowing us to focus attention on the precursor of OSCC. Moreover, aberrant miRNAs are involved in both chronic inflammation and cancer initiation. OLP is a unique disease model of inflammation-associated cancer for studying both chronic inflammation and OSCC.[9,12] This short report attempts to evaluate whether aberrantly expressed salivary microRNAs can serve as feasible biomarkers for screening OPMD progression and detecting early OSCC. This analysis indicates that aberrant salivary miRNAs as noninvasive biomarkers for OPMD onset and progression represent a new paradigm in the clinical application of the disorders.

Salivary diagnostics has attracted significant attention among clinicians and investigators because the method of sample collection for disease is noninvasive, simple, repeatable, rapid, and cost-effective. Importantly, oral epithelial cells, e.g. oral potentially malignant cells, are continually immersed in the salivary milieu. Aberrant miRNAs are present in saliva, and the release of miRNAs into the saliva is explained as a result of necrotic or apoptotic cell death and also by active cell secretion. The reconstruction of the miRNA profile in the saliva is in contact with oral epithelial cells. Accordingly, oral biofluid/saliva may provide direct information regarding the disease status of oral mucosa. It could be a fascinating alternative when biopsy specimen is insufficient for further processing, and makes it striking option for diagnosing, monitoring and prognosis of various oral diseases. This noninvasive procedure could be proposed not in replacement of invasive biopsy for definite diagnosis but as an easily performed and effective measure of oral cancer risk to alert healthy providers and general dentists.

Although salivary miRNAs may be a promising noninvasive biomarker for the diagnosis and risk assessment of OPMD, there still existed some obvious limitations. First, limited studies reported salivary miRNAs in OPMD, and the great mass of the investigations were comparative case—control studies with very small sample size. Also, few longitudinal follow-up cohorts focused on OPMD progression to OSCC. Secondly, few studies have come up with area under the curve (AUC) with sensitivity and specificity characteristics in OPMD patients as diagnostic or predictive analysis. The available data on the values of AUC, sensitivity and specificity are presented in Fig. 1. Thirdly, the included studies on over 20 miRNAs adopted diverse cutoff values for miRNA quantification. The standardized protocols for salivary collection, storage along with RNA

extraction are also needed to lessen the discrepancies across different studies. Besides, salivary miRNAs as markers are hindered by the lack of consensus on the internal control being used for analysis.

In summary, aberrant salivary miRNAs, especially miR-21 and miR-31, were associated with onset and progression of OPMD, and could then serve as noninvasive biomarkers for screening OPMD and detecting malignant changes. However, multiple combined markers should be discovered and developed, and large-scale longitudinal study design should be launched to commit surveys acquiring the extraordinary disease prediction.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jds.2022.08.002.

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