Review

MicroRNAs as biomarkers for dental diseases

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\textbf{A R T I C L E I N F O}

Keywords:
- microRNA
- Biomarker
- Saliva
- Periodontitis

\textbf{A B S T R A C T}

MicroRNAs (miRNAs) are short, noncoding RNAs that act as key regulators of diverse biological processes by mediating translational repression or mRNA degradation of target genes. Recent studies discovered miRNAs in saliva, and these miRNAs are promising candidates for use as biomarkers of dental diseases. In this review, the results of miRNA studies in the dental field are presented, and a brief overview of the current progress, limitations, and perspectives regarding miRNA biomarkers for dental diseases is given.

\textsuperscript{2015} Published by Elsevier B.V.

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MicroRNAs

MicroRNAs (miRNAs) are a class of small single-stranded noncoding RNAs that were first discovered in \textit{C. elegans} and later shown to be evolutionarily conserved across many animal species, including humans \cite{1-3}. MicroRNAs, which are approximately 22 nucleotides in length, act as key regulators of diverse biological processes by mediating translational repression or mRNA degradation of their target genes \cite{4,5}. The mode of action of these regulators is through imperfect complementary binding to the 3' untranslated region (3' UTR) of target mRNAs \cite{4,6}. Typically, a single

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http://dx.doi.org/10.1016/j.sdj.2015.09.001
0377-5291/\textsuperscript{2015} Published by Elsevier B.V.
miRNA has the potential to simultaneously control the translation of hundreds of genes [5,7]. To date, more than 2500 genes encoding miRNAs have been identified in the human genome [8,9].

The biogenesis of miRNAs begins with the production of mRNA-like polyadenylated primary transcripts (pri-miRNA) by RNA polymerase II. Subsequent miRNA maturation requires two RNase III proteins, Drosha and Dicer. These two proteins may collaborate in the stepwise processing of miRNAs, and also have key roles in the process of miRNA-mediated gene regulation. The mature single-stranded miRNAs are eventually incorporated into a ribonucleoprotein complex, the RNA-induced silencing complex (RISC). RISC acts to downregulate gene expression via mRNA cleavage or translation repression: the miRNA component acts as a guide [10–14].

### Circulating microRNAs

Serum and plasma contain large amounts of stable miRNAs, and these therefore have potential to serve as biomarkers for specific physiological and pathological conditions such as cancer, transplant rejection, cardiac injury, infection, and others [3,15–18]. The salivary transcriptome, which contains more than 3000 RNA species, including miRNAs, was recently released, and salivary miRNA biomarkers are emerging as tools for the detection of oral cancer and systemic diseases [19–23]. The miRNAs in saliva have advantages for biomarkers compared with other salivary biomarkers like proteins, miRNAs, DNAs and bacterial products. Even aside from the distinctive function of miRNA as post-transcriptional regulator, miRNAs are stably present in saliva and the similarity between miRNA profiles of saliva and other body fluids provide high availability as biomarkers for various human diseases [21,24,25].

Saliva sampling is a non-invasive, cheap, easy-to-access alternative to traditional tissue and blood sampling [26,27]. Saliva is an accurate indicator of bodily conditions, and salivary biomarkers may serve as early diagnostic tools. Saliva diagnostics is considered to be a highly promising alternative to classic environmental epidemiology [28,29]. The ease of sampling and cost effectiveness of saliva-based tests provide advantages over traditional techniques for large scale population-based screening studies and also in situations where repeated sampling is required, such as for monitoring and managing disease progression [30]. Lab-on-a-chip (LOC) technologies are beginning to be used in clinical settings for point-of-care (POC) diagnostics [31]. Saliva samples have been used successfully for POC detection of multiple disease entities, including some that cause dental disease [32,33].

### MicroRNAs as biomarkers for oral cancers

MicroRNAs are important in tumourigenesis due to their proximity to chromosomal breakpoints and their dysregulated expression levels in many malignancies [34–36]. Overexpression of certain miRNAs might result in the downregulation of tumour suppressor genes, while underexpression of other miRNAs might cause oncogene upregulation [37,38]. Consequently, several studies evaluated the potential of miRNAs as diagnostic and prognostic biomarkers for cancers [20,24,29,40].

Approximately 90% of head and neck cancers are oral squamous cell carcinomas (OSCC). OSCC is an aggressive and lethal malignancy that is a major worldwide problem due to its extremely high prevalence and very poor prognosis [41,42]. The average 5-year survival rate for patients with diagnosed OSCC is approximately 50% [43,44]. Therefore, an early detection method is needed for OSCC to improve treatment and long-term patient survival [20]. A number of studies have evaluated miRNAs as diagnostic biomarkers for OSCC. Differentially expressed miRNAs discovered by these studies are listed in Table 1.

### MicroRNAs as biomarkers for periodontitis

Periodontal disease is characterised by inflammation of tooth-supporting structures. Periodontitis, which is a typical manifestation of periodontal disease, involves progressive loss of the alveolar bone around the teeth. If left untreated, periodontitis can lead to tooth loosening and subsequent tooth loss [52–55]. Associations between periodontal disease and other general health problems, such as diabetes, cardiovascular disease, rheumatoid arthritis and adverse

#### Table 1 - Differential expression of miRNAs in oral cancers (Human model).

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Sample source</th>
<th>Disease</th>
<th>Expression</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-211</td>
<td>Tissue</td>
<td>OSCC</td>
<td>Increased</td>
<td>Chang et al. [45]</td>
</tr>
<tr>
<td>miR-125a, miR-200a</td>
<td>Saliva</td>
<td>OSCC</td>
<td>Increased</td>
<td>Park et al. [20]</td>
</tr>
<tr>
<td>miR-21, miR-155, let-7i, miR142-3p, miR-423, miR-106b, miR-20a, miR-16</td>
<td>Tissue</td>
<td>HNSCC</td>
<td>Decreased</td>
<td>Hui et al. [46]</td>
</tr>
<tr>
<td>miR-125b, miR-375, miR-10a</td>
<td>Plasma</td>
<td>OSCC</td>
<td>Increased</td>
<td>Lin et al. [47]</td>
</tr>
<tr>
<td>miR-74</td>
<td>Plasmas</td>
<td>OSCC</td>
<td>Increased</td>
<td>Liu et al. [22]</td>
</tr>
<tr>
<td>miR-31</td>
<td>Tissue</td>
<td>HNSCC</td>
<td>Decreased</td>
<td>Chen et al. [48]</td>
</tr>
<tr>
<td>miR-99a, miR-100</td>
<td>Saliva</td>
<td>HNSCC</td>
<td>Increased</td>
<td>Salazar et al. [49]</td>
</tr>
<tr>
<td>miR-9</td>
<td>Tissue</td>
<td>HNSCC</td>
<td>Decreased</td>
<td>Ren et al. [50]</td>
</tr>
<tr>
<td>miR-134, miR-191</td>
<td>Whole blood</td>
<td>OSCC</td>
<td>Increased</td>
<td>Shi et al. [51]</td>
</tr>
<tr>
<td>miR-21</td>
<td>Tissue</td>
<td>OSCC</td>
<td>Increased</td>
<td>Momen-Heravi et al. [38]</td>
</tr>
<tr>
<td>miR-155</td>
<td>Saliva</td>
<td>OSCC</td>
<td>Increased</td>
<td>Momen-Heravi et al. [38]</td>
</tr>
<tr>
<td>miR-27b</td>
<td>Saliva</td>
<td>OSCC</td>
<td>Increased</td>
<td>Momen-Heravi et al. [38]</td>
</tr>
</tbody>
</table>

* Head and neck squamous cell carcinoma (HNSCC), oral squamous cell carcinoma (OSCC).

miRNA is a naturally occurring small RNA molecule that functions as a post-transcriptional regulator, playing a crucial role in various biological processes such as cell growth, differentiation, and apoptosis. The miRNA expression profile can be used as a diagnostic tool for the detection of various diseases, including oral cancers and periodontitis. Saliva sampling is a non-invasive and cost-effective method for miRNA analysis, making it a promising alternative to traditional tissue and blood sampling.
MicroRNA research related to periodontal disease.

<table>
<thead>
<tr>
<th>Target diseases</th>
<th>Sample source</th>
<th>Methods</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periodontitis</td>
<td>Healthy and diseased gingival tissues</td>
<td>miRNA PCR array</td>
<td>6 miRNAs up-regulated</td>
<td>Lee et al. [64]</td>
</tr>
<tr>
<td>Periodontitis</td>
<td>Healthy and diseased gingival tissues</td>
<td>miRNA Microarray</td>
<td>91 miRNAs up-regulated, 34 miRNAs down-regulated</td>
<td>Xie et al. [58]</td>
</tr>
<tr>
<td>Periodontitis and obesity</td>
<td>Gingival biopsy samples</td>
<td>miRNA PCR array</td>
<td>11 miRNAs up-regulated</td>
<td>Perri et al. [59]</td>
</tr>
<tr>
<td>Periodontitis</td>
<td>Healthy and diseased gingival tissues</td>
<td>miRNA Microarray</td>
<td>4 miRNAs up-regulated, 7 miRNAs down-regulated</td>
<td>Stoecklin-Wasmer et al. [61]</td>
</tr>
</tbody>
</table>

Limitations of miRNAs as biomarkers

Although the exploitation of miRNAs as biomarkers for dental diseases is promising, some constraints should be considered.

First, differentially expressed candidate miRNAs identified through pilot studies need to be validated. Most previous studies using tissues were performed with samples from small numbers of individuals without matching for potential confounding factors known to influence periodontitis susceptibility such as age and gender. Further validation studies using large well-characterised cohorts are required [60].

Second, although recent advances in molecular biology and high-throughput screening techniques have enabled researchers to characterise miRNA patterns in body fluids such as serum, plasma, and saliva on a large scale, this is limited by the lack of suitable endogenous controls for normalisation of salivary miRNAs. Recent research attempted to identify endogenous control miRNAs displaying minimal expression variability between samples [38], but endogenous salivary miRNA controls are required for future exploitation of miRNA datasets. In addition, saliva samples collected from the same individual can display considerable heterogeneity according to the collection method used, and standardised methods for sample collection should therefore be considered.

Third, although biomarker development is paramount, the development of suitable treatment and prevention methods for patients testing positive for these biomarkers is also required. Effective, focused treatments are needed for highly susceptible patients in order to capitalise on early diagnosis, and without such treatments a clear cost-benefit advantage cannot be realised [28].
Future directions

The availability of novel genetic testing technologies such as RNA sequencing will allow current technical limitations to be circumvented and will increase test accuracy when extremely small samples are used. Fundamental research into the mechanisms underlying control and activity of miRNAs will facilitate the identification of links between various diseases. In addition, more efficient computational prediction models and improved bioinformatic pipelines will allow optimal use of miRNA datasets [65–67]. The combination of new diagnostic tests with saliva sampling will provide easy, rapid, testing, and will enable large scale and follow-up studies to be conducted at lower costs than when using traditional blood or tissue samples. With the introduction of new and improved techniques, more individuals susceptible to periodontal disease and with poor prognosis for other conditions will be detected early, allowing patient-focused clinical treatments. This first step will lay the groundwork for the future development of personalised dentistry using genetic information.

Acknowledgements

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (NRF-2013R1A1A2011974).

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