Alterations in miRNA Expression Patterns in Whole Blood of OSCC Patients

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Abstract. Background/Aim: Aberrant microRNA (miRNA) expression in blood of cancer patients is a common finding. The present study aimed at evaluating the differences in miRNA expression in whole blood samples of oral squamous cell carcinoma (OSCC) patients compared to healthy controls. Materials and Methods: Microarray based miRNA profiling was performed on whole blood samples of 20 OSCC patients and 20 healthy volunteers and the differences in expression patterns between the two groups were evaluated. The results were validated by Reverse Transcription quantitative-Polymerase Chain Reaction (RT-qPCR) in 50 OSCC patients and 35 volunteers. Results: 21 miRNAs were identified to be significantly differentially expressed in whole blood of OSCC patients compared to healthy controls. The impact of miR-186 (p=0.002), miR-494 (p=0.001) and miR-3651 (p=0.0001) could be validated by RT-qPCR. Conclusion: The aberrant expressions of miR-186, miR-494 and miR-3651 in whole blood of OSCC patients may serve as the basis for establishing minimally-invasive screening methods for OSCC based on miRNAs as biomarkers.

The prognosis of oral squamous cell carcinoma (OSCC) is poor (1). The high mortality is based on the absence of suitable parameters for prognostic assessment, clinical monitoring of the disease and for choosing the most appropriate therapy option for each individual patient (2). Aberrant expression of various genes could provide information on those parameters (3).

MicroRNAs are approximately 22 nucleotides (nt) long non-coding single-stranded RNA molecules. They inhibit gene expression by multiple mechanisms including base-specific interaction with the target mRNA transcripts. They are essential regulators of many cellular processes, including cell cycle, apoptosis, cell migration, and many pathological processes including cancer (4). They have been shown to be de-regulated in virtually all types of cancers and differential miRNA expression profiles could also be demonstrated in specimens of OSCC compared to matched healthy controls. In addition, these global alterations in miRNA expression levels have been associated with important aspects of cancer development. Hence, miRNAs represent a promising class of cancer biomarkers (5-10).

miRNAs are stable in blood and altered miRNA expression patterns could be confirmed in blood of patients suffering from different kinds of solid tumors when compared to healthy persons. Consequently, it seems that miRNAs can be considered as potential minimally invasive diagnostic markers for the detection and prognosis of malignant diseases (11-17). Differential expression of miRNAs has also been shown in plasma or serum of patients suffering from head and neck squamous cell carcinoma (HNSCC) (18, 19). Unfortunately, only for a few studies the tumors were located exclusively in the oral cavity (10, 20-23). However, HNSCC is a heterogeneous group including tumors of the oral cavity, the nasal cavity, esopharynx, pharynx and larynx and the biology of the malignancy differs significantly depending on tumor localisation. Consequently, expression patterns of miRNAs also differ based on tumor localisation (9, 24). Hence, the results are not necessarily transferable on OSCC. Another shortcoming of the former studies is that only one of the blood components, either plasma or serum, was obtained for investigations, although miRNA profiling based on whole blood seems to be the most reliable one (17). Furthermore, only single miRNAs were examined and the analyzed aberrantly expressed miRNAs were selected a priori based on studies of genomic...
investigations or expression profiles of miRNAs in cancer tissues. However, there is evidence that miRNAs showing disease-associated changes in blood are not necessarily the same ones to be differentially expressed in cancer tissues (20, 21, 23, 25). Moreover, only one study dealing with high-risk oral lesions examined the expression of a large number of miRNAs in serum using miRNA microarray (26). However, the first step in identifying more miRNA candidates is the expression analysis by miRNA microarrays which allows for investigation of a large number of different miRNAs at the same time.

The aim of this study was to compare the miRNA expression patterns in whole blood of patients suffering from OSCC with healthy controls using miRNA microarrays in order to classify those alterations in the expression rate of particular miRNAs. Thus, miRNAs were identified that could be potential biomarkers. To our best knowledge, this is the first global analysis of miRNA expression profiles by microarrays in whole blood samples of patients suffering exclusively from OSCC. The results might provide new insights into the molecular basis of OSCC and might identify biomarkers for minimally-invasive diagnosis, therapy, prognosis evaluation and tumor monitoring in the future.

Materials and Methods

Patients and sample collection. Whole blood samples of 50 OSCC patients (test group) and 33 age-matched healthy volunteers (control group) were collected. The study was approved by the Ethics Committee of the University of Erlangen-Nuremberg, Erlangen, Germany. Patients’ informed consent was obtained. Patients were eligible for inclusion into the study if OSCC occurred for the first time and if whole blood samples could be collected before surgical removal of the tumor or radiotherapy and/or chemotherapy. TNM classification was evaluated according to International Union Against Cancer (27). OSCC was also characterized according to the World Health Organization (WHO) for loss of differentiation as G1, G2 and G3 for well, moderately and poor differentiation, respectively. All biopsies were classified by two pathologists to ensure consistent results. Tumors were also grouped as early (including stage I and II) and late (including stage III and IV) clinical stages. Furthermore the lymph node status was grouped as N=0 and N>0 in order to indicate cases with negative and positive lymph node status, respectively. Healthy controls were selected based on the absence of general disease and acute or chronic inflammations.

This study was divided into two steps. In step 1, for miRNA microarrays screening, preoperative whole blood from 20 OSCC patients (test group) and blood samples from 20 age-matched healthy individuals (control group) were collected (Microarray cohort of test and control group). miRNA profiles were generated from whole blood of OSCC patients and controls and then compared with each other. Thus, the most differentially expressed miRNAs were identified and subsequently examined in further studies (step 2).

In step 2, for RT-qPCR screening, whole blood samples were collected from additional 30 OSCC patients and 15 healthy volunteers (RT-qPCR validation cohort of t-test and control group). Expression rates of the conjunctural miRNAs identified in step 1 were compared between the two groups in order to confirm the relevance of the identified miRNAs.

Sampling of peripheral blood, miRNA isolation and quality control. Two and a half ml whole blood of OSCC patients and healthy volunteers were collected in duplicates in a Paxgene Blood RNA Tube (PreAnalyticX, Hombrechtikon, Switzerland). The samples were carefully inverted in order to mix miRNA supplied “stabling puffer” with the blood, incubated at room temperature for two hours and stored at −80°C until miRNA isolation.

Whole RNA was extracted using PAXgeneBlood miRNA Kit as recommended by the provider (PreAnalyticX, Hombrechtikon, Switzerland). RNA concentration was measured with a NanoDrop spectrometer (PeqLab, Erlangen, Germany). The integrity and size distribution of total RNA used in microarray investigation was additionally checked by using the Agilent 2100 bioanalyzer (Agilent Technologies Inc., Santa Clara, CA, USA) and the RNA 6000 Nano Kit (Agilent Technologies, Waldbronn, Germany). Subsequently, the miRNA samples were stored at −80°C.

miRNA microarray expression analysis. miRNA microarrays were performed on the Geniom® real-time analyzer (GRTA, Febit, Heidelberg, Germany) using the Agilent’s SurePrint G3 Human v16 miRNA Array Kit, 8x60K (Release 16.0) microarrays which were updated from the Sanger miRBase 16.0 in September 2010 (Agilent Technologies, Inc, Santa Clara, CA, USA). The miRNA Microarrays included 1205 human and 144 viral human miRNAs. For biotinylation and hybridisation, Agilent’s miRNA Complete Labeling and Hybridization Kit was used. After hybridisation for 16 h at 42°C, the biochip was washed automatically and a program for signal enhancement was processed with the GRTA. The resulting detection pictures were evaluated using the Geniom® Wizard Software (Febit GmbH, Heidelberg, Germany). Then, for each array and each miRNA (feature), the median signal intensity was extracted from the raw data file so that for each miRNA seven intensity values were calculated corresponding to each replicate copy of miRBase on the array. Following background correction via the Feature Extraction Software from Agilent, median values were calculated from the replicate intensity values of each miRNA. To normalize the data across different arrays, quantile normalization was applied and all further analyses were carried out using the normalized and background subtracted intensity values.

Real-time Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR) expression analysis. Five miRNAs (let-7d*, mir-186, -494, -3162, -3651) which were identified by miRNA microarray assays and verified by Volcano Plot to be differentially expressed were analyzed in an independent validation cohort of test (n=50) and control group (n=35) by RT-qPCR. The analyses were performed on 500 ng of total RNA. In the first step, miRNA was reverse- transcribed using the miScript II RT Kit according to the manufacturer’s recommendation (Qiagen, Hilden, Germany). Detection of amplification was done on 2.5 ng of cDNA with SYBR green nucleic acid stain on an ABI-7300 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using the miScript SYBR Green PCR Kit and miRNA specific quantitative RT-PCR primer sets for the miRNA of interest (Qiagen). The features of the miRNAs are summarized in Table I.
The values of RT-qPCR analyses were normalized by the ΔΔCT method. For that purpose the primer sets RNU6-2 (U6 snRNA, RNA U6 small nuclear 2) and SNORD44 (small nucleolar RNA, C/D box 44), which are indicated to be stably expressed in whole blood across normal and cancer patients, were taken as internal controls (Qiagen, Hilden, Germany, Table I). The mean value of both controls was applied as normalization value. Relative quantification of differences in expression (RQ=2−ΔΔCT) between the two groups was done by the ΔΔCT method using Microsoft EXCEL® 2003 for Windows (Microsoft Corporation, Redmond, USA).

Statistical analyses. (A) Statistics for miRNA microarray: The study was focused on the miRNA expression differences between whole blood samples of patients compared to those of normal blood samples. For this purpose the following different statistical measurements were applied, after the normal distribution of the measuring data had been checked by the test of Shapiro-Wilk: parametric t-test (unpaired, two-tailed), Wilcoxon Mann-Whitney U-test (WMW, unpaired, two-tailed), a linear model with p-values computed by an empirical Bayes approach (Limma), the area under the receiver operator characteristics curve (AUC) and determination of the fold change quotients. All p-values were adjusted for multiple testing by Benjamini–Hochberg (28) adjustment.

p-Values of limma less than 0.05 and expression signals showing at least a 2-fold difference in abundance between tumor and control samples were considered as statistically significant. AUC values can range from 0 to 1. A value of 0.5 indicates equal distribution among healthy and diseased subjects and means that the intensity values generated by RNA from blood of patients and healthy subjects cannot be used to make a distinction between both groups. An AUC value above 0.5 means higher expression intensities of the respective miRNA in OSCC samples (up-regulated miRNA) whereas an AUC under 0.5 means higher expression values of the miRNA in controls (down-regulated miRNA). An AUC of 1 and 0 corresponds to a perfect separation.

Additionally, hierarchical clustering via the 30 most differentially expressed miRNAs was carried-out to identify samples that show similar patterns of intensity value and thus are similar to each other. A heatmap was generated showing a color representation of samples and probes ordered by their similarity with dendrogram on the top (clustering of samples) and on the right (clustering of probes).

At last the discriminatory accuracy of the deregulated miRNAs were displayed by performing a Volcano Plot for comparison of the control vs. carcinoma group by taking into account the changes in expression (log fold changes) and the statistical significance of the change in log odds [quotients from the probability that an event occurs and the probability that it does not occur (complementary probability)]. By this way the five most deregulated probes were verified. (B) Statistics for RT-qPCR: In this study, relative quantification was applied, and thus appropriate internal normalization controls (RNU6-2 and SNORD44) were chosen to normalize sample-to-sample variations. For statistical evaluation of RT-qPCR analysis the program IBM® SPSS statistics 19 (Chicago, IL, USA) was applied. In addition relative expressions (RQ) of the examined genes between the two groups were calculated by the ΔΔCT method using the formula (RQ=2−ΔΔCT) taking into account the mean values of all ΔΔCT values within a group.

Two fold changes of miRNA expression rates (2≤RQ≤0.5) between the two groups were defined as statistically relevant.

The mean value of duplicate ΔΔCT values of each sample was used for the data results. Expression data were controlled for normal distribution by the Shapiro-Wilk test. Data derived from RT-qPCR and presented as ΔΔCT values were expressed as the median (ME), the interquartile range (IQR), standard deviation (SD), and range. Graphical diagrams are plotted as Box-Whisker Plots which represent the median, the interquartile range, minimum and maximum values of determined miRNA expression. Statistical relevance of the apparent expression between the two groups was analyzed by the Mann-Whitney U-test. p-Values ≤0.05 were considered as statistically significant.

Furthermore, the expression profile of each differentially expressed miRNA was used for creation of receiver operator characteristics (ROC) curves. This method displays the discriminatory accuracy of the marker for distinguishing between two groups. The area under the ROC curve (AUC) of miRNAs defines the usefulness of a miRNA with respect to its ability to separate the two different groups of blood donors.

Results

Characteristics and clinico-histopathological parameters of the study participants. For miRNA expression profiling by microarray 20 blood samples of OSCC patients and the

| Table I. List of miRNAs and endogenous controls analyzed by miScriptPrimerAssay. |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| ID                         | Sanger accession           | Sequence                    | Order number (Qiagen)       |
| Hsa-Let-7d-5p*             | MIMAT000065                 | AGAGGUAGUAGGUUGCAUGAGU       | MS 00008302                 |
| Hsa-miR-186-5p             | MIMAT0000456                | CAAAGAAUCUCCUUUUUGGCCU       | MS 00008883                 |
| Hsa-miR-3651               | MIMAT0018071                | CAUAGCGCGGUGCUAUCAGA         | MS 00023121                 |
| Hsa-miR-3162-5p            | MIMAT0015036                | UUAGGGAGUAGAAGGUUGGGAG       | MS 00020762                 |
| Hsa-miR-494-5p             | MIMAT0026607                | AGGUUGUCGGUUGUCUCUC         | MSC 0002535                  |

Endogenous controls

<table>
<thead>
<tr>
<th>Ref Seq</th>
<th>Order number (Qiagen)</th>
</tr>
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<tbody>
<tr>
<td>RNU6-2</td>
<td>NR_002752</td>
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<tr>
<td>SNORD44</td>
<td>NR_002750</td>
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control group (microarray cohorts) were examined. The demographical and histopathological characteristics of the study’s participants are summarized in Tables II and III. For the validation set, 35 blood samples were collected for the control group. Demographic characteristics are shown in Table III. None of the healthy volunteers has got remarkable oral mucosal changes, like inflammation and hyperplasia or dysplasia. In the validation cohort of test group, 50 OSCC patients were included. Demographic characteristics and clinico-histopathological parameters of patients suffering from OSCC are summarized in Table II. There were no significant differences between the microarray cohorts of test and control group as well as between the validation cohorts of the groups for any listed parameter if compared by the Mann-Whitney U-test (p>0.05).

Identification of OSCC-associated miRNAs in whole blood by miRNA microarray. The first step of our study was the identification of potential miRNAs for diagnostic application by large scale expression analyses. For this purpose miRNA expression profiling was performed and the two differential miRNA expression patterns were created. Afterwards these patterns were compared between OSCC and normal blood.

Out of the 1,205 tested human miRNAs, several miRNAs were differentially expressed in patients when compared to the control group. In the hierarchical cluster analysis clusters of up- and down-regulated miRNAs in blood of OSCC patients vs. healthy controls were documented. Their patterns clearly separate the two groups (Figure 1).

The 21 most de-regulated miRNAs were identified by determination of fold changes and statistical evaluation by t-tests and empirical Bayes approaches (limma) followed by Benjamini–Hochberg adjustment. The results are summerized in Table IV. For all genes the AUC value was significant and amounted on higher than 0.80 for up-regulated and lower than 0.2 for all down-regulated miRNAs (Table IV).

Subsequently, out of the determined 21 candidates the 5 statistically most relevant miRNAs were verified by performing a Volcano Plot (Figure 2). As shown in Table IV miR-186 and let-7d* were down-regulated approximately two- and 4-fold in the test group, respectively. The highest fold change was observed for miR-494. This miRNA was expressed approximately 5-fold higher in cancer patients. The up-regulation was statistically relevant (p=0.02) and the AUC value amounted on 0.82. The abundance of miR-3162 was 2.1-fold and the one of miR-3651 was 2.5-fold higher in the blood of OSCC patient. The limma p-values revealed that the changes were statistically significant (p=0.003, p=0.02). The usefulness of the miRNAs with respect to their ability to separate the two different groups of blood donors is also demonstrated by the AUC values lying about 0.8 and under 0.2, respectively (Table IV).

RT-qPCR screening for miRNA markers. In step 2, the five conjectural miRNAs identified in step 1 were validated by real-time RT-qPCR. These analyses were performed on 50 blood samples from OSCC patients and on 35 samples from healthy controls (validation cohort). For further statistical analysis all data are presented as ΔCT values. The results
are graphically plotted as Box-Whisker plots which represent the median (ME), the interquartile range (IQR), standard deviation (SD) and range as well as the minimum and maximum values of ΔCT values. The fold changes are given as RQ values which were determined by the ΔΔCT method and calculated by the formula 2-ΔΔCT (Figure 3).

With regards to the results of the statistical investigation among the five most differentially expressed miRNAs there were no significant differences in expression between OSCC and healthy controls for let7d* (RQ=0.82, p=0.2) and miR-3162 (RQ=1.2, p=0.601) (Figure 3). In addition, the determined AUC values were not significant (p=0.59, p=0.5) (Figure 4B). The expressions of the remaining three miRNAs, miR-186, miR-3651 and miR-494 were significantly different in OSCC patients' blood compared to healthy controls with higher ΔCT values standing for
lower miRNA-expression. MiR-186 was significantly reduced in the patients’ group. It was down-regulated two-fold and the \( p \)-value for differential expression was 0.002. The expression of the two other miRNAs was significantly elevated. The fold change for miR-3651 was two-fold whereas this value was 2.3 for the miR-494. The \( p \)-values were 0.0001 and 0.001, respectively (Figure 3). In order to confirm the statistical relevance of the markers a ROC (Receiver Operating Characteristic) curve was established and AUC was determined. The up-regulated miRNAs yield an AUC of 0.82 and 0.72, respectively (Figure 4A). The AUC value of miR-186 accounts to 0.69 (Figure 4A).

**Discussion**

Since the discovery of stable miRNAs in whole blood, serum and plasma and their differential expression in healthy individuals and patients, circulating miRNAs have been shown to be powerful biomarkers for diagnostic investigation and even for cancer management and direct monitoring of disease using a minimally-invasive method (11, 12, 24, 29). The aim of the present study was to analyze the miRNA expression patterns in whole blood of patients suffering from OSCC in comparison to healthy controls using miRNA microarrays and to validate the identified markers by RT-qPCR.
Twenty-one miRNAs were identified to be significantly de-regulated in OSCC when the expression patterns in whole blood samples of tumor patients were compared to healthy controls. It could be shown that the different expression patterns of these miRNAs allow the differentiation between these two groups. The five most differentially expressed ones were confirmed by a Volcano Plot. Three of these (miR-3162, miR-3651, miR-494) were up-regulated and two (miR-3162, let-7d*) down-regulated. The relevance of these miRNAs could be validated by RT-qPCR and it seems that they may be useful for establishing a minimally-invasive blood-based diagnostic and prognostic method for OSCC.

The possible application of miRNAs in clinical tools is based upon their influence on a plenty of cancer-relevant biological pathways and many of them might be important for OSCC development and progression. This way, the ubiquitously expressed miRNA let-7d plays an important role during embryonic development and can prevent tumorigenesis. Its expression is frequently found to be down-regulated in tissues of several types of tumor including HNSCC (19, 30). Furthermore, miR-186 has been reported to be disease-associated. Thus, miR-186 is involved in inhibition of invasion when overexpressed in cancer tissue samples and could be an anti-invasion target for therapeutic intervention for non-small cell lung cancer (31). Moreover, down-regulation in esophageal cancer and lung adenocarcinoma was associated with poor prognosis by interference of this miRNA with cell-cycle regulation genes (32). Additionally, miR-186 may induce cellular senescence and regulate apoptotic response (33, 34). On the basis of the identified functions it could be concluded that the altered expression of miR-186 might also play a critical part in oral carcinogenesis and prognosis. Therefore, this miRNA might be an important marker for diagnosis, prognosis and therapy in OSCC. miR-494 has been reported to act as tumor suppressor by inducing cell-cycle arrest, cell senescence, apoptosis and by suppressing cell proliferation. Furthermore, it is down-regulated in different kinds of cancer tissues (35-37). However, we found a higher level of this miRNA in the blood of tumor patients. This contradictory result may be due to selective exosome-mediated release of the miRNA into the extracellular environment. The exclusion of the miRNA from cellular matrix leads to loss of its tumor-suppressive function and consequently to cancer cell phenotypes (26). Function and involvement of the human specific miRNAs, miR-3651 and miR-3162, in cancer development and progression are not investigated till now. Further studies are necessary to ascertain the impact of these markers in malignancies including OSCC.

Recently, a number of differentially expressed miRNAs in blood of patients suffering from HNSCC have been identified and are discussed to be potential blood biomarkers also for OSCC. Particularly, the circulating miRNAs, miR-24, miR-31 and miR-184, have been intensely studied, and their power in the clinical monitoring of disease is already suggested. (8, 10, 20, 21, 29). However, we were not able to confirm their differential expression. This may be due to several reasons. Firstly, only a small collection of circulating miRNAs which were pre-selected by their occurring deregulation in cancer tissues was evaluated in former studies (10, 20, 21). However, miRNAs showing disease-associated expression changes in blood are not necessarily the same ones that are differentially expressed in cancer tissues and aberrant expression might not correlate (25). Secondly, all studies were performed by RT-qPCR and not by miRNA microarray. However, discrepancy in aberrant expression profiles between the two groups gained by the different methods was already observed in other studies (38). Furthermore, in former studies the differential miRNA expression was evaluated either in plasma or serum of OSCC patients and not in whole blood. However, differential miRNA expression was shown in patient-matched serum and
plasma samples. Additionally, significant differences in miRNA profiles between cell-free and cellular blood were reported (11, 14).

Today quantitative RT-PCR is the gold standard in expression profiling of miRNAs. Hence, expression data from microarray experiments were confirmed for the five most de-regulated miRNAs in an independent OSCC and control group by this method. The significant down-regulation of miR-186 and the up-regulation of miR-3651 and miR-494 could be shown in whole blood samples of OSCC patients. Hence, the detection of their aberrant expression in blood could allow discrimination between healthy controls and OSCC patients and, consequently, the detection of OSCC. However, the aberrant expression of let-7d and miR-3162 could not be confirmed. This discrepancy in expression profiles gained by different methods was already observed in other studies and the low correlation between the two methods indicates considerable variability between the two assay platforms (38). Moreover, it has been considered that microarray-based gene expression analyses often show decreased sensitivity and reliability. In contrast, a high reproducibility was demonstrated for RT-qPCR. Moreover, a higher false-positive rate of differential miRNA expression was observed using the microarray and PCR indicated superior sensitivity and specificity of RT-PCR (38). Hence, the results of our PCR analyses seem to be the more reliable ones. Nevertheless, further studies using other primers for PCR or methods like various hybridization

![Figure 3. RT-qPCR validation of miRNAs. Legend: Box-Whisker-plots of median expression rates of the miRNAs in whole blood of OSCC and control group derived from RT-qPCR and given as median ΔCT values. Higher CT values stand for lower miRNA expression. The median, the interquartile range and the standard deviation are indicated. Relative Quantification (RQ) was done by the ΔΔCT method comparing the average ΔCT values of OSCC vs. control group and indicates the fold change. Statistical evaluation was done by the Mann-Whitney U-test. The expression was statistically relevant changed in OSCC vs. control for miR-186, miR-3651 and miR-494.](image-url)
Figure 4. Area under the receiver operating characteristic curve (AUC) for the miRNAs based on the RT-qPCR. Legend: The diagram is a plot of the sensitivity (true positive rate) versus 1-specificity (false positive rate) over all possible ΔCT values. (A) ROC of miRNAs which were significantly changed. The AUC values indicate that the two groups could be distinguished by expression analysis of the markers. (B) miRNAs which show no significant AUC value.
techniques including Northern blotting are necessary to dissolve this discrepancy of the results gained by the two former methods.

Circulating miRNAs as blood-based biomarkers for OSCC seem to be a useful tool for establishing a minimally-invasive method for OSCC detection, screening and monitoring. Thus, recent studies using serum or plasma for RT-PCR based miRNA-profiling were promising and today several miRNAs have been identified as potential serum/plasma biomarkers in different types of cancer including OSCC (29). Moreover, evaluated post-surgical concentrations of different miRNAs in plasma samples of OSCC patients were associated with recurrence and predict risk of worse clinical outcome (10, 20-22). Indeed, for these applications the techniques based on whole blood seem to be the most reliable ones (17). The miRNAs identified by us could also be useful for such applications. However, further validation in a larger cohort is required to assess the utility of the miRNAs as oral cancer biomarkers. Additionally, the impact of the identified miRNAs in clinical monitoring, prediction of recurrence and prognosis has to be evaluated in the future by follow up studies like it is described for other tumor types especially for lung cancer (17, 39-42).

Conclusion

The aberrant expressions of miRNAs especially of miR-186, miR-494 and miR-3651 in whole blood of OSCC patients may serve as the basis for establishing these miRNAs as minimally invasive biomarkers for the detection of OSCC. They may also be useful as additional clinical prognostic factors in predictive models of OSCC recurrence and clinical monitoring.

Conflicts of Interest

The Author(s) declare that they have no competing interests.

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