Prognostic significance of altered miRNA expression in whole blood of OSCC patients

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Abstract. Currently, there is a lack of blood markers for the detection of recurrent oral squamous cell carcinoma (OSCC). The present study aimed to investigate whether the aberrant expression of single microRNAs (miRNAs) in whole blood of patients could serve as a biomarker for persistent or recurrent OSCC. Whole blood of 2 groups of formerly treated OSCC patients was investigated by RT-qPCR for their circulating miRNA profiles. The R-OC group included patients with recurrence of OSCC (n=21) and the NR-OC group included patients without recurrence (n=21). Fold-changes and significance of the differences in miRNA expression levels between the groups were determined. A cut-off point (COP) for the discrimination between the R-OC and NR-OC groups was calculated and the significance between over/under expression of the miRNAs and the recurrence of malignancy was determined. Significant differences in the miRNA expression in whole blood of the R-OC and NR-OC groups were found. The levels of miR-3651 and miR-494 were significantly increased and the level of miR-186 was significantly decreased in whole blood of the R-OC patients (pmiR-3651=0.001, pmiR-494 =0.003 and pmiR-186=0.001). By the determination of the COP, increased or decreased expression of the markers was significantly correlated to the recurrence of the disease. Altered expression of miR-494, miR-3651 and miR-186 appears to be associated with the recurrence of OSCC. The present study may form the basis for establishing a blood test as a minimally invasive method for the detection of the recurrence of OSCC.

Introduction

Late diagnosis, frequent regional lymph node metastases and the high rate of local and regional recurrence that is caused mainly by the persistence of malignant cells, are the major causes for the poor prognosis of oral squamous cell carcinoma (OSCC) (1,2). Recurrence leads to a significantly decreased life expectancy of the affected patients (3). Early detection of recurrence improves patient survival (4-7).

TNM classification, histological grade and the depth of tumor invasion are the best known prognostic factors for recurrence, even if the real prognostic value of these clinical and pathological features is still controversial (2,8). Additionally, the state of the margins after tumor resection has been investigated in order to predict recurrence. However, even following wide tumor excision, a local recurrence of OSCC occurs in up to 30% of OSCC patients due to residual malignant cells that could not be microscopically detected in tumor margins at the time of surgery (9).

To solve these shortcomings of the clinical parameters, there is an urgent need to determine highly sensitive and tumor-specific biomarkers for the stratification of patients with high risk of disease recurrence and for long-term surveillance of OSCC as well as for restaging procedures to exclude or confirm tumor recurrence at stages that allow a successful therapeutic intervention (10). Unfortunately, intensive research spanning several decades has failed to identify new prognostic molecular markers in oral cancer. To date, relevant prognostic indicators with sufficient sensitivity and specificity are nonexistent (11). Therefore, the importance of developing useful diagnostic and monitoring tools is emphasized in order to improve the clinical outcome of patients suffering from OSCC.

MicroRNAs (miRNAs) are predicted to control ~50% of all gene expression (12). Thus, altered miRNA expression has been associated with several diseases including the development of different types of cancers (13,14). In addition, miRNAs are released from cells and enter the circulation, where they are highly stable. They have been found in several human body fluids, including plasma, serum, whole blood cells and saliva (15). The alterations in cancer cells are thought to be reflected in the extracellular space as affected cells release upregulated miRNAs, fail to release apparently downregulated species or export selectively the type of miRNAs which suppress tumorigenesis.

Therefore, the detection of altered patterns of circulating miRNAs in the blood has been proposed as cancer biomarkers used in minimally invasive assays that may aid in risk
assessments, diagnosis, prognosis, detection of recurrence, monitoring of the clinical course of the disease, and in predicting or monitoring treatment response as well as in therapy decision making in various types of cancer (11,16-24) including OSCC (25-27). Ultimately, it has been proven that alterations in the patterns of miRNAs predict recurrence risk and are associated with therapeutic response (28-30). Recently, we demonstrated that the miRNAs, miR-494, miR-3651 and miR-186, are differentially expressed in whole blood of OSCC patients when compared to that of healthy volunteers. The altered expression rates were significantly correlated to diagnosis. Additionally, overexpression of miR-3651 was associated to lymph node metastases, correlated to diagnosis. Additionally, overexpression

### Materials and methods

#### Patients and sample collection

The present study was approved by the Ethics Committee of the University of Erlangen-Nuremberg (Erlangen, Germany) (approval no. 3962) and patient written informed consent was obtained.

Patients who suffered from a primary OSCC in the past were considered to be free of tumor cells after surgical removal of the tumor. The patients were followed up for 1 year at time-points of 1, 3, 6, 9 and 12 months after primary treatment in order to detect recurrence of disease. Based on these observations 2 groups were established. The R-OC group included 21 OSCC patients who suffered from recurrence compared to patients who were disease-free for at least 1 year after treatment in order to clarify their impact in the early detection of recurrent disease.

#### Statistical analysis

For statistical evaluation, IBM® SPSS Statistics 21 (SPSS, Inc., Chicago, IL, USA) was used. The mean value of duplicate ΔCT values for each sample was used for the data results. Expression data were controlled for normal distribution using the Shapiro-Wilk test. Graphical diagrams are plotted as Box-Whisker plots which represent the median (ME), the interquartile range (IQR) and the standard deviation (SD) as well as the minimum and maximum values. The mean value of duplicate ΔCT values for each sample was used for the data results. Expression data were controlled for normal distribution using the Shapiro-Wilk test. Graphical diagrams are plotted as Box-Whisker plots which represent the median (ME), the interquartile range (IQR) and the standard deviation (SD) as well as the minimum and maximum values.

#### Real-time quantitative reverse transcription-PCR (RT-qPCR)

The values of the miRNAs, miR-186, miR-494 and miR-3651, were determined by RT-qPCR. These analyses were carried out 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 recommendations (Qiagen, Hilden, Germany). Detection of amplification was carried out on 2.5 ng of cDNA using the miScript SYBR-Green PCR kit and miRNA-specific quantitative RT-PCR primer sets for the miRNA of interest (Qiagen) were determined on an ABI-7300 Sequence Detection System (Applied Biosystems, Foster City, USA). The features of the miRNAs are summarized in Table II.

#### Sampling of whole blood and miRNA isolation

Two aliquots of whole blood (2.5 ml) for each subject (tumor, R-OC, NR-OC and control group) were collected in a PAXgene Blood RNA Tube (PreAnalyticX, Hombrechtikon, Switzerland) and stored at -80°C until RNA isolation.

Whole RNA was extracted using the PAXgene Blood miRNA kit as recommended by the manufacturer. The RNA concentration was assessed using NanoDrop spectrometer (PeqLab, Erlangen, Germany), and sufficient quality of the samples for RT-qPCR analysis was confirmed using A260/A280 ratios. Subsequently the RNA samples were stored at -80°C.

#### Statistical analysis

For statistical evaluation, IBM® SPSS Statistics 21 (SPSS, Inc., Chicago, IL, USA) was used. The mean value of duplicate ΔCT values for each sample was used for the data results. Expression data were controlled for normal distribution using the Shapiro-Wilk test. Graphical diagrams are plotted as Box-Whisker plots which represent the median (ME), the interquartile range (IQR) and the standard deviation (SD) as well as the minimum and maximum values.

### Table I. Demographic characteristics of the test (OSCC patients), control (healthy volunteers) and follow-up groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Test group</th>
<th>Control group</th>
<th>R-OC group</th>
<th>NR-OC group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>54</td>
<td>33</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Mean age ± SD (years)</td>
<td>65.04±10.97</td>
<td>60.5±20.68</td>
<td>60.43±8.25</td>
<td>62.1±12.96</td>
</tr>
<tr>
<td>Age range (years)</td>
<td>35-93</td>
<td>15-88</td>
<td>51-83</td>
<td>33-81</td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td>Male 37 (68.5)</td>
<td>23 (69.7)</td>
<td>16 (76.2)</td>
<td>14 (66.7)</td>
</tr>
<tr>
<td></td>
<td>Female 17 (31.5)</td>
<td>10 (30.3)</td>
<td>5 (23.8)</td>
<td>7 (33.3)</td>
</tr>
</tbody>
</table>

R-OC, tumor patients suffering from a recurrence within 1 year; NR-OC, patients with no recurrence within 1 year. SD, standard deviation.

### Table II. miRNA expression in whole blood of OSCC patients when compared to that of healthy volunteers.

<table>
<thead>
<tr>
<th>miRNA</th>
<th>ΔCT values for each sample was used for the data results. Expression data were controlled for normal distribution using the Shapiro-Wilk test. Graphical diagrams are plotted as Box-Whisker plots which represent the median (ME), the interquartile range (IQR) and the standard deviation (SD) as well as the minimum and maximum values.</th>
<th>ΔΔCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-3651</td>
<td>2-fold change in miRNA expression rate</td>
<td>0.5 ≤ RQ ≤ 2</td>
</tr>
<tr>
<td>miR-186</td>
<td>2-fold change in miRNA expression rate</td>
<td>0.5 ≤ RQ ≤ 2</td>
</tr>
<tr>
<td>miR-494</td>
<td>2-fold change in miRNA expression rate</td>
<td>0.5 ≤ RQ ≤ 2</td>
</tr>
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R-OC, tumor patients suffering from a recurrence within 1 year; NR-OC, patients with no recurrence within 1 year. SD, standard deviation.
Table II. List of miRNAs, snRNAs (endogenous controls) and miScript Primer Assay used in the RT-qPCR analysis.

<table>
<thead>
<tr>
<th>Sanger ID</th>
<th>Sanger accession</th>
<th>Mature miRNA</th>
<th>Sequence</th>
<th>Ref. no. (Qiagen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>miRNAs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hsa-miR-186-5p</td>
<td>MIMAT0000456</td>
<td>CAAGAAUUCCUCUUUGGCU</td>
<td>MS 00008883</td>
<td></td>
</tr>
<tr>
<td>hsa-miR-3651</td>
<td>MIMAT0018071</td>
<td>CAUAGCCCGGUUGCUAGA</td>
<td>MS 00023121</td>
<td></td>
</tr>
<tr>
<td>hsa-miR-494-5p</td>
<td>MIMAT0026607</td>
<td>AGGGUGCCCGUUUGUCUCU</td>
<td>MSC 0002535</td>
<td></td>
</tr>
<tr>
<td>Endogenous controls</td>
<td>Ref. Seq.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNU6-2</td>
<td>NR_002752</td>
<td></td>
<td></td>
<td>MS 00033740</td>
</tr>
<tr>
<td>SNORD44</td>
<td>NR_002750</td>
<td></td>
<td></td>
<td>MS 00007518</td>
</tr>
</tbody>
</table>

*Custom miScript Primer Assay.

maximum values of the ΔCT values. Statistical relevance of the apparent expression between the 2 groups was analyzed using 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 operating characteristics (ROC) curves, and for estimation of the area under the curve (AUC). This method displays the discriminatory accuracy of the marker for distinguishing between the 2 groups of blood donors.

Using ROC curves the highest Youden index (Y) was calculated. The Youden index is associated with the critical expression point or the optimal threshold value respectively [named cut off-point (COP)] for the biological marker. The COP indicates which value of increased or decreased expression is relevant for the discrimination between malignancy and normal samples and allows assignment of a particular sample to a certain group.

Based on these COPs the 2 groups were divided into 2 subgroups which exhibited an expression rate over or under the COP. Afterwards, association between altered miRNA expression and malignancy, clinical features and histopathological parameters were calculated using the Chi-square test.

**Results**

Demographic characteristics of the study participants. Whole blood samples were collected from 54 OSCC patients (tumor-group), 33 healthy volunteers (control group), 21 patients who relapsed after primary treatment within 1 year, and from 21 patients who did not suffer from a recurrence for at least 1 year after primary treatment. The demographical characteristics of all study participants are summarized in Table I. None of the healthy volunteers had marked oral mucosal pathologies, such as inflammation, hyperplasia or dysplasia. All groups matched in regards to gender and age. There were no statistically relevant differences determined by the Mann-Whitney U test. The R-OC and the NR-OC groups matched in regards to gender and age (P=0.5; P=0.359), respectively.

**RT-qPCR screening for miRNA expression differences between all groups.** A significantly different expression of all assessed miRNAs could be ascertained between the OSCC and control group (p_{miR-186}=0.012; p_{miR-3651}=0.001; p_{miR-494}=0.007) and the NR-OC group (p_{miR-186}=0.001; p_{miR-3651}=0.0001; p_{miR-494}=0.039), respectively. In contrast there was no significant difference in the expression values between the OSCC and the R-OC group (p_{miR-186}=0.94; p_{miR-3651}=0.89; p_{miR-494}=0.21). The comparison of the expression values between the control and the NR-OC group revealed no statistical relevant discrepancy (p_{miR-186}=0.07; p_{miR-3651}=0.41; p_{miR-494}=0.45), while the expression of the miRNAs significantly varied when the control group was compared to the patients who developed recurrence (R-OC group) (p_{miR-186}=0.03; p_{miR-3651}=0.0001; p_{miR-494}=0.003). A statistically significant difference in expression rate could also be assigned when the recurrence group (R-OC) was compared to the patients who did not develop a recurrence (NR-OC group) within 1 year (p_{miR-186}=0.03; p_{miR-3651}=0.001; p_{miR-494}=0.003). The results of the statistical evaluation are summarized in Table III.

**RT-qPCR screening for miRNA expression between the R-OC and the NR-OC group.** The impact of the differences in the expression levels of the miRNAs for the discrimination between the NR-OC and the R-OC groups was more accurately evaluated statistically in order to assess their usefulness for the detection of recurrence and the clinical monitoring of the disease. A significantly different expression could be determined between the NR-OC and the R-OC group for miR-186, miR-3651, and miR-494 (Table IV; Fig. 1). The results are graphically plotted as Box-Whisker plots. Higher ΔCT values signify lower miRNA expression levels. RQ values indicate the changes in the expression levels (Table IV; Fig. 1).

miR-186 was significantly decreased in whole blood when recurrence occurred (P=0.003). It was downregulated 2.1-fold. The expression of the two other miRNAs was significantly increased. The fold-change for miR-3651 was 2-fold whereas this value amounted to 2.3 for miR-494. The increased expression was significantly associated to recurrence (p_{miR-3651}=0.001; p_{miR-494}=0.003). The results are presented in Fig. 1 and Table IV.

Between the R-OC and the NR-OC group an ROC curve was established and the AUC was determined. All markers yielded a significant AUC value. The upregulated miRNAs, miR-3651 and miR-494 yielded an AUC of 0.80 and 0.78, respectively. The AUC value of the decreased miR-186 amounted to 0.76 (Fig. 2; Table IV).
The highest Youden indices were 0.476 for miR-494, 0.525 for miR-3651 and 0.524 for miR-186 (Table IV). The optimal threshold values/cut-off points (COP) expressed as a ΔCT value were 5.87 for miR-494, 1.16 for miR-3651 and 13.39 for miR-186. For the miRNAs miR-494 and miR-3651 a ΔCT under the COP (signifying upregulation) was considered to be positive for the recurrence corresponding to an increased abundance of the marker in whole blood. For miR-186 a ΔCT value over the COP (signifying downregulation) was positive for recurrence. Using the determined COPs the 2 groups were divided into positive and negative specimens. The changes in the expression rates of the miRNAs were statistically relevant and associated to recurrence. The results are diagrammed in Fig. 3 and summarized in Table V.

Out of the patients with recurrence 71.4% (15/21) exhibited decreased levels of miR-186 and 71.4% (15/21) and...
76.2% (16/21) exhibited increased values of miR-3651 and miR-494, respectively. Only 14.3% (3/21) of the whole blood samples from the NR-OC group exhibited decreased levels of miR-186. Increased levels in blood of patients suffering from NR-OC were revealed in 19.0% (4/21) of the cases for miR-3651 and in 23.8% (5/21) for miR-494, respectively. In order to ascertain the utility of the combination of 2 miRNAs as prognostic tools 3 additional arrangements of the investigated miRNAs were made. When a combination of miR-186/miR-494 and miR-186/miR-3651 was assessed 100% of the R-OC specimens exhibited positivity for altered expression, whereas only 42.9 and 38.1% of the NR-OC samples showed positivity. When, using the combination miR-494/miR-3651 85.7% of the patients with recurrence were positive, whereas only 33.3% of the patients with no recurrence exhibited specific altered expression of 1 of these 2 markers. The correlation between recurrence and the detection of altered expression rates was significant for all the investigated miRNAs and their various combinations (P<0.01). Moreover, 100% of the blood samples of patients suffering from recurrence exhibited altered expression of at least 1 of the examined miRNAs whereas in only 52.4% of the disease-free patients such an altered abundance was evident. Thus, the association to recurrence was statistically relevant (P=0.0001).

The sensitivity of miRNAs, miR-186, miR-3651 and miR-494 amounted to 0.714, 0.714 and 0.762, respectively. The values of the specificity of miR-186, miR-3651 and miR-494 were 0.857, 0.81 and 0.762. The analyzed combinations reached different values for sensitivity: miR-186/miR-494, 1; miR-186/miR-3651, 1 and miR-494/miR-3651, 0.857; and specificity: miR-186/miR-494, 0.571; miR-186/miR-3651, 0.619 and miR-494/miR-3651, 0.667.

**Discussion**

Approximately 50% of patients with OSCC present with metastatic disease or local recurrence at the time of initial diagnosis or in the aftercare, leading to an unfavorable...
prognosis (38). Therefore, early detection of primary and/or recurrent OSCC by routine laboratory tests is required (4,5,7). Moreover, the monitoring of the response to successful therapy by serial assessment of biomarkers, showing different pretreatment and post-treatment levels is much-warranted (39). Tumor biomarkers in blood and saliva may allow an earlier detection of the primary and/or recurrent disease, and may serve as possible predictors of prognosis for OSCC. Unfortunately, most of the identified markers lack the required specificity and sensitivity. Therefore, the importance of developing useful diagnostic and monitoring tools is emphasized in order to increase initial recognition of patients with a higher risk of recurrent disease and to improve long-term clinical outcomes by allowing for a more aggressive treatment approach and by carrying out a closer follow-up of these patients in the aftercare (8).

miRNAs are important regulatory molecules and are shown to be involved in disease pathogenesis. The perceived opportunity for their use as clinical markers has loomed particularly large in neoplastic disease, where alterations in cancer cells are thought to be reflected in the extracellular space as affected cells release upregulated miRNAs or fail to release apparently downregulated species (18). Additionally, miRNAs are very stable and can therefore be detected in several body fluids including serum, plasma, saliva and whole blood. Several studies have demonstrated the usefulness of circulating miRNAs as potential biomarkers that may aid in risk assessment, diagnosis, prognosis, and monitoring of disease and of treatment response for OSCC (25-27,33,34,40-47).

In the present study the hypothesis that differential expression of defined miRNAs in whole blood samples of OSCC patients could be associated with recurrence of OSCC and can therefore predict recurrent disease and provide clinically relevant prognostic information for OSCC patients in a post- operative setting was assessed.

In previous studies, we identified 3 differentially expressed miRNAs in whole blood of OSCC patients when compared to that of healthy volunteers (31,32). The miR-494 and miR-3651 concentrations in OSCC patients were increased, whereas miR-186 exhibited decreased abundance. The altered expression rates of these miRNAs were significantly associated to diagnosis. Additionally, overexpression of miR-3651 was significantly correlated to lymph node metastasis, clinical stage and to more dedifferentiated tumors. Thus, it was postulated that these miRNAs may be useful in prognostic applications and may serve as the basis for establishing a minimally invasive method for the detection and monitoring of OSCC (31,32).

In the present study, we confirmed the significant changes in the expression of the investigated miRNAs in OSCC patients compared to healthy controls. The diagnosis was significantly correlated to the detection of altered expression rates of all examined miRNAs. In addition, the changes in miRNA concentrations were significantly different between healthy people and patients with recurrence (R-OC), as well as in OSCC patients compared to patients with no recurrence (NR-OC), respectively. In contrast, these miRNAs were not differentially expressed in whole blood of volunteers when compared to patients with no recurrence or in whole blood of patients suffering from a primary OSCC compared to test subjects who relapsed. These results strengthen the hypothesis that the occurrence of primary as well as of recurrent malignancies can be diagnosed by the expression analysis of these miRNAs in whole blood.

It has already been demonstrated that increased postsurgical concentrations of different miRNAs in plasma, serum and saliva samples of OSCC patients were associated with recurrence and predicted worse clinical outcome (46-49). In the present study, we revealed a significantly different expression of miR-186, miR-3651 and miR-494 in whole blood between the group of patients with recurrence and those with no recurrence that allows for the discrimination between the 2 groups. Moreover, taking into account the COPs the presence of upregulated miR-494 and miR-3651 and downregulated miR-186 in individual pro-bands was significantly correlated with the absence and presence of the recurrence of the disease. Moreover, the markers showed high sensitivity and specificity, and promising positive or negative predictive values. Thus, it appears that these 3 miRNAs are relevant biomarkers in the differentiation of patients with recurrence.

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**Table V. Association between altered expression rates of each individual miRNA and recurrence.**

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Positive n/%</th>
<th>P-value</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Predictive value (Positive/negative)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R-OC</td>
<td>NR-OC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>186-5p</td>
<td>15/71.4</td>
<td>3/14.3</td>
<td>0.001</td>
<td>0.714</td>
<td>0.857</td>
</tr>
<tr>
<td>3651</td>
<td>15/71.4</td>
<td>4/19.0</td>
<td>0.001</td>
<td>0.714</td>
<td>0.81</td>
</tr>
<tr>
<td>494-5p</td>
<td>16/76.2</td>
<td>5/23.8</td>
<td>0.002</td>
<td>0.762</td>
<td>0.762</td>
</tr>
<tr>
<td>186/494a</td>
<td>21/100</td>
<td>9/42.9</td>
<td>0.0001</td>
<td>1</td>
<td>0.571</td>
</tr>
<tr>
<td>186/3651a</td>
<td>21/100</td>
<td>8/38.1</td>
<td>0.0001</td>
<td>1</td>
<td>0.619</td>
</tr>
<tr>
<td>494/3651a</td>
<td>18/85.7</td>
<td>7/33.3</td>
<td>0.001</td>
<td>0.857</td>
<td>0.667</td>
</tr>
<tr>
<td>Totalb</td>
<td>21/100</td>
<td>11/52.4</td>
<td>0.0001</td>
<td>1</td>
<td>0.476</td>
</tr>
</tbody>
</table>

Positivity of the blood samples of the particular patients was ascertained using the determined COP of each respective miRNA. R-OC (n=21), OSCC patients suffering from a recurrence within 1 year; NR-OC (n=21), patient without recurrence within 1 year. Value of statistical relevance of the association was determined by the Chi-square test. *Combination of two different miRNAs; bat least 1 out of the examined miRNAs.
to those with no recurrence. Thus, they could be useful for the establishment of a minimally invasive method based on blood assessment that may provide an early indication of the existence of persistent or recurrent OSCC in an individual patient. Furthermore, it was also revealed that all patients with recurrence were positive for at least 1 differentially expressed miRNA. Thus the sensitivity of this multimarker combination was excellent. However, 52.4% of the patients with no recurrence were determined as positive. This led to a low degree of specificity. Additionally, the paired combination of the 2 markers resulted in a higher sensitivity of the test, but in contrast in a concomitant loss of specificity. Therefore, in the future it may be useful to explore more than 1 biomarker in order to enhance the diagnostic accuracy (8,50). However, by establishing combinations of markers both the sensitivity and specificity of the test may be investigated and the ratio between these 2 values may be taken into consideration in order to achieve methods with optimal sensitivity as well as the highest possible specificity.

Besides the detection of recurrent disease, many issues could be addressed toward clinical application. For several tumors including OSCC numerous potential miRNAs as valuable biomarkers in predicting the behavior of individual cancers and monitoring therapeutic responses could be displayed. Studies on various tumors evaluating blood miRNAs as biomarkers for OSCC management have been carried out (27,34,43-46,51,52). Moreover, these identified markers may also be applied in the risk assessment of precancerous lesions (53). These studies led to the formulation of the phrase ‘liquid biopsy’ that may allow for the early warning of oncogenesis, relapse and treatment failure (18). Consequently, our results may encourage the expansion of studies on the evaluation of blood miRNAs as biomarkers for OSCC management and monitoring of the disease. However, further validation in a larger cohort is warranted to fully assess the utility of particular miRNAs as OSCC biomarkers. Additionally, further investigations are warranted for the screening of other miRNAs for clinical use which have already been shown to be valuable tools for these clinical applications. Moreover, their impact in clinical monitoring and prognosis has to be evaluated by particular prospective follow-up studies. Finally in order to translate the promising results into clinical practice these markers have to be validated in well-designed and sufficiently powered multi-centre studies.

In conclusion, altered levels of miR-494, miR-3651 and miR-186 which are differentially expressed in primary OSCC patients were also observed upon disease relapse. Thus, the present study clearly demonstrated the usefulness of circulating miRNAs in the screening of OSCC patients and for the early detection of recurrent and persistent disease. This may provide us with the possibility of setting up a minimally invasive method for OSCC patient stratification according to the risk of recurrence, clinical monitoring and therapeutic response in order to optimize the treatment of patients and to improve tumor outcome.

Acknowledgements

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References