ABSTRACT
Oral squamous cell carcinoma (OSCC) is the most common cancer type arising in the oral cavity and belongs to the wider group of head and neck cancers (HNCs). MicroRNAs (miRNAs) in mature form are noncoding RNAs, that regulate posttranscriptional gene expression by relatively nonspecific binding. As a result, a single miRNA may regulate/control the expression of several genes which are involved in a variety of cellular processes like regulation of cellular differentiation, proliferation and apoptosis thus playing a role in cancer development. The aim of this review was to summarize current findings on the effect of HPV status and presence of risk factors - smoking and alcohol consumption on miRNA expression as cancer biomarkers in patients with OSCC.

KEYWORDS: oral cancer, OSCC, miRNA expression, HPV, smoking, alcohol

Introduction
Oral squamous cell carcinoma (OSCC) is the most common cancer type arising in the oral cavity and belongs to the broader group of head and neck cancers (HNCs). It is the sixth most common cancer in humans [1], accounting for about 24% of all HNCs [2]. The five-year survival rate for patients with stage I OSCC is 80% and substantially decreases with higher stages to approximately 40% [3], [4], [5]. No reliable biomarkers are available to detect OSCC aggressiveness and predict disease outcome to allow personalized therapies for individual tumors [6]. Molecular and cellular biology is a promising field of study that may lead to the discovery of novel biomarkers and novel therapeutic targets. The study of microRNAs as such biomarkers and their role in the molecular biology of human diseases has been an area of intense research. With the discovery of microRNA molecules in Caenorhabditis elegans in 1993 [7], a novel method of gene expression regulation was revealed [8]. MicroRNAs (miRNAs) in mature form are noncoding RNAs, 19 to 25 nucleotides in length, that regulate posttranscriptional gene expression by relatively nonspecific binding [9]. As a result, a single miRNA may monitor/control the expression of several genes. It has been proposed that over one-third of all protein-coding genes be under translational control by miRs (premature miRNAs) [10], which are involved in a variety of cellular processes like regulation of cellular differentiation, proliferation and apoptosis thus playing a role in cancer development [11]. Global expression analysis in different cancers has identified miRNAs that function as oncomirs, like miR-21-5p, which are consistently upregulated in some cancer types [12]. Other miRNAs are reduced in spe-
Table 1 MicroRNA profile of OSCC.

| miRNAs   | Upregulated / Downregulated | References          |
|----------|----------------------------|---------------------|
| miR-21   | Upregulated                | [39][40][41][42][43][44][45][46][47][48][49][50] |
| miR-31   | Upregulated                | [51][52][43][44][45][54][55][56][46][57][41][50][58] |
| miR-184  | Upregulated                | [46][59][46][60][39] |
| miR-211  | Upregulated                | [61][62] |
| miR-196  | Upregulated                | [63][64][56][65][39][66][67] |
| Let-7 family | Up or downregulated | [42][68][69] |
| MiR-375  | Downregulated              | [70][71][44] |
| miR-155  | Upregulated                | [69][70][74][75] |
| miR-99   | Downregulated              | [76][77][78][46][79][67] |

The risk of developing oral and oropharyngeal cancer (OPC) increases with age, and the majority of cases occur in people aged 50 or over. Risk factors include smoking, chewing tobacco, snuff dipping, alcohol misuse and HPV infection [1]. Although oral cavity and oropharyngeal cancers are strongly related to smoking [18], other detrimental risk factors, such as alcohol [19] and human papillomavirus (HPV) [20] may substantially modify the trends.

Based on substantial evidence from numerous observational studies, tobacco and alcohol use are well-known risk factors for the development of oral cavity and oropharyngeal cancer and account for 75% of the disease burden of oral and oropharyngeal malignancies in Europe, the Americas and Japan [30]. Oral cavity cancer incidence rates increased in many countries with tobacco epidemics that are currently peaking and declined in areas where tobacco use peaked some time ago [31]. Smoking and alcohol consumption are independently and synergistically associated with an increased risk of oral cancer [32]. The risk for current smokers is about tenfold that of never-smokers and is dose related. Most cancers of the oral cavity are attributable to the use of tobacco products [33][34][35]. Alcohol itself has a lower effect than that associated with tobacco use, but the risk is approximately doubled for people who drink three to four alcoholic beverages per day compared with nondrinkers and is dose related [23][36][37]. The relationship between years of tobacco smoking and alcohol consumption and cancer risk also followed an intense, dose-response relationship overall. Quitting the bad habits significantly reduced cancer risk after at least three years of cessation [38]. Oropharyngeal cancer incidence increased significantly from 1983 to 2002 predominantly in developed countries and in younger patients which underscored a potential role for HPV infection for increasing OPC incidence, particularly among men [21]. A recent analysis of oropharyngeal squamous cell carcinomas (OPSCCs) estimated that the proportion of OPSCC attributable to HPV infection increased from 16% to 72% between 1988 and 2004 in contrast to a 50% decline in the incidence of HPV-negative cancers [22].

On the other hand, the association of oral cavity cancers and human papillomavirus is heterogeneous. HPV is an established cause of OPC (including the tonsil, base of the tongue, and other parts of the pharynx) whereas its etiologic role in OSCC is unclear [25][26][27]. According to a systematic review HPV, DNA was detected in 24% of OCSCC worldwide. However, the presence of HPV DNA alone is insufficient evidence for a causal association from a molecular perspective. Expression of HPV oncogenes E6 and E7 remains a gold standard for classification of HPV-caused cancer and is necessary for tumour initiation [28]. In one recent study, the HPV type distribution observed in a series of OSCCs was significantly more diverse than for the corresponding series of oropharyngeal cancers (94.9% HPV16, 5.1% non-16) [29] with approximately 38% of positive cases attributable to high-risk HPV types other than 16. Studies that focus exclusively on HPV16 may, therefore, underestimate associations. The International Agency for Research on Cancer (IARC) recently estimated the HPV-attributable fraction for OCSCC to be 5% of the 263,000 annual cases of OCSCC resulting in a total of 13,150 oral cancer cases worldwide [20].

This review aimed to summarise current findings on the effect of HPV status and presence of risk factors - smoking and alcohol consumption on miRNA expression as cancer biomarkers in patients with OSCC. As specific data on oral cancer was scarce, comparison with data on oropharyngeal cancer was considered to be relevant and worthwhile discussing.

**miRNA profile of oral squamous cell carcinoma**

More and more studies in recent years focused on miRNA deregulation in OSCC. To determine a miRNA profile of OSCC multiple and independent studies have been reviewed where candidates were investigated for their role in oral tumorigenesis, as potential biomarkers, prognostic factors and therapeutic agents. Different tumour types have also revealed myriad oncogenic miRNAs, but some stand out as specific for OSCC pathogenesis and take part in the development or progression of the disease (Table 1).

MiR-21 is one of the most well-studied miRNAs, and it has a clear role in the carcinogenesis of various malignant tumours. MiR-21 is overexpressed in both OSCC and HNSCC [39][40]. Multivariate analysis showed that miR-21 expression is an independent prognostic factor indicating poor survival [41]. MiR-21 serves as a chemosensitive miRNA as its expression was found to be lower in a cisplatin-resistant tongue squamous cell line.
Table 2: Risk factors and their effect on miRNA expression in OSCC and HNSCC.

| Risk Factor | Differentially expressed miRNAs | Effect of risk factor presence on miRNA expression | Study | Cancer type | References |
|-------------|--------------------------------|-----------------------------------------------|-------|-------------|------------|
| HPV         | miR-195-star                    | up                                            | In vivo | Oropharyngeal SCC (HPV+ vs HPV-) | Lajer et al 2011 |
|             | miR-363                         | up                                            |       |             |            |
|             | miR-127-3p                      | down                                          |       |             |            |
|             | miR-379                         | down                                          |       |             |            |
|             | miR-125a-5p                     | down                                          |       |             |            |
|             | miR-432                         | down                                          |       |             |            |
|             | miR-143                         | down                                          |       |             |            |
|             | miR-145                         | down                                          |       |             |            |
|             | miR-409-5p                      | down                                          |       |             |            |
|             | miR-433                         | down                                          |       |             |            |
|             | miR-381                         | down                                          |       |             |            |
|             | miR-199a-3p                     | down                                          |       |             |            |
|             | miR-199b-3p                     | down                                          |       |             |            |
|             | miR-26b_st                      | down                                          |       |             |            |
|             | miR-199b-5p                     | down                                          |       |             |            |
|             | miR-143-star                    | down                                          |       |             |            |
|             | miR-1201                        | down                                          |       |             |            |
|             | miR-126                         | down                                          |       |             |            |
|             | miR-409-3p                      | down                                          |       |             |            |
|             | miR-101                         | down                                          |       |             |            |
|             | miR-517a                        | down                                          |       |             |            |
|             | miR_363                         | up                                            | In vitro | SCCHN cell lines (HPV-16+ vs HPV-16-) | Wald et al 2011 |
|             | miR_497                         | up                                            |       |             |            |
|             | miR_33                          | up                                            |       |             |            |
|             | miR_155                         | down                                          |       |             |            |
|             | miR_181a                        | down                                          |       |             |            |
|             | miR_181b                        | down                                          |       |             |            |
|             | miR_29a                         | down                                          |       |             |            |
|             | miR_218                         | down                                          |       |             |            |
|             | miR_222                         | down                                          |       |             |            |
|             | miR_221                         | down                                          |       |             |            |
|             | miR_142_5p                      | down                                          |       |             |            |
|             | miR_13232                       | down                                          |       |             |            |
| Alcohol     | miR-30a                         | up                                            | In vivo | HNSCC       | Saad et al 2015 |
|             | miR-934                         | up                                            |       |             |            |
|             | miR-3178                        | up                                            |       |             |            |
|             | miR-675                         | up                                            |       |             |            |
|             | miR-101                         | up                                            |       |             |            |
|             | miR-1266                        | up                                            |       |             |            |
|             | miR-3164                        | up                                            |       |             |            |
|             | miR-3690                        | up                                            |       |             |            |
|             | miR-34a                         | down                                          | In vivo | Oral SCC    | Mariakandan et al 2015 |
| Smoking     | miR-637                         | up                                            | In vivo | Oral SCC    | Kolokythas et al. 2015 |
compared to a chemosensitive tongue cancer cell line [42]. The putative oncogene expression was consistently increased and associated with increases in lesion severity during progression and also played an important role in malignant transformation [39]. MiR-21 was consistently found in various studies ([43][44][45][46][47][48][49][50]) related to oral cancer indicating its major role in the malignant transformation and progression of the lesion.

miR-31 was reported to be upregulated in OSCC and also plasma miR-31 in patients was remarkably reduced after tumour resection suggesting that this marker was tumour-associated [51]. miR-31 could be validated as a marker of OSCC for diagnostic uses because the feasibility of detecting its increase in patient’s saliva was also demonstrated. A recent study showed that upregulation of both miR-31 and miR-31* by delivery of pre-miR-31 enhanced OSCC oncogenicity [52]. Detection of miR-31 expression is an adjuvant method for the screening of high-risk oral potentially malignant disorder (OPMD) and patients with recurrent OPMD and malignant transformation exhibited a further augmented expression of miR-31 [43]. Expression of miR-31 was significantly elevated in early stage tumours with no metastatic nodes and those from buccal mucosa [44] and was also found upregulated in oral premalignant epithelium [53]. Just like miR-21 there are numerous studies associating miR-31 with oral carcinoma [45][46][53][54][55][56][57][58].

Some miRNAs such as miR-184 have been identified as deregulated in OSCCs accurately and not in other head and neck squamous cell carcinoma types. Inhibition of miR-184 in tongue SCC cell lines would reduce cell proliferation rate and suppress it would induce apoptosis in cell lines which defines its oncogenic role in the antiapoptotic and proliferative processes of tongue SCC [46]. Furthermore, when compared with pre-surgical levels, miR-184 levels were considerably lower following tumour resection [59]. Additionally, the plasma miR-184 levels were significantly higher in patients with TSCC when compared with healthy individuals, and this level decreased considerably after the surgical removal of a primary tumour [46], [60]. When studying the progression of leukoplakia to OSCC miR-184 was found to be an early detectable miRNA in oral tumour progression [39].

miR-211 is predicted to target multiple genes, including genes involved in angiogenesis and apoptosis. An association was found between higher miR-211 expression and advanced nodal metastasis, vascular invasion, and poor prognosis of oral carcinoma [61]. Enforced miR-211 expression significantly increased the proliferation, migration, and anchorage-independent colony formation of oral carcinoma cells [61]. The miR-211 expression may be associated with the progression of oral carcinoma and poor patient outcomes [61]. miR-211 could facilitate HNSSC progression via the inhibition of TGFβRII, disrupted Smad3 phosphorylation and upregulated c-Myc expression [62].

The miR-196 family consists of two members, miR-196a and miR-196b, which share sequence similarity but are transcribed from three different genes. There is only one nucleotide difference between mature miR-196a and miR-196b, indicating that they possess similar cellular functions and share common regulatory networks [63]. Some studies identified that miR-196 might interact with several transcription factors and be involved in cancer development and progression [63][64]. Significant up-regulation of miR-196 was found in oral cancer cell lines [56]. MiR-196 has also upregulated in OSCC tissue relative to control mucosa with high expression of miR-196a. It was associated with tumour recurrence, nodal metastasis, and mortality - the risk of mortality was most significant for patients with high miR-196a levels and positive node status [65]. Cervigni et al. found it was overexpressed in OSCC, but underexpressed in dysplasias and proposed final stages of carcinogenesis may also involve changes in miR-196a [39]. In a clinical study, both circulating miR-196a and miR-196b were substantially upregulated in patients with oral pre-cancer lesions (5.9- and 14.8-fold), as well as in oral cancer patients (9.3- and 17.0-fold). These results showed prominent discrimination between normal and pre-cancer patients and between healthy and cancer patients suggesting the combined determination of miR-196a and miR-196b levels produced excellent sensitivity and specificity in the diagnosis of patients with oral pre-cancer or oral cancer, as well as in the prediction of potential malignancy [66]. When the sensitivity and specificity for miR-196a/196b were tested in general, miR-196a provided excellent specificity for diagnosing pre-cancer (94%), cancer (96%) and potential malignancy (96%), but was less sensitive for screening these diseases (56-67%). By contrast, miR-196b provided superb sensitivity for screening pre-cancer (100%), cancer (98%) and potential malignancy (93%), but was less specific for diagnosing these diseases (58-81%) [66]. Kolokythas et al. stressed the importance of miR196a-5p in OSCC malignancy in both never and ever smokers in the overall similarity of miRNA expression in OSCCs in these two risk groups [67].

The Let-7 family is a family of tumour suppressor miRNAs that regulate multiple oncogenic pathways. The increased levels of let-7c, -7d, -7e, -7g were found in the Tca/cisplatin cells comparing with the Tca8113 cells, suggesting that they might play a role in the chemoresistance against cisplatin in Tca/cisplatin cells [42]. Compared with healthy primary gingival epithelial cells elevated expression levels of Dicer in oral cancer cells correlated with down-regulation of let-7b and increased cell proliferation. ROC analysis of the 20 miRNA candidates identified five miRNAs including let-7b that have an AUC >0.8, suggesting that these miRNAs could have utility as biomarkers for oral cancer detection [68]. Christensen et al. reported reduced let-7 miRNA levels in oral tumours relative to pharyngeal neoplasms and pointed that cases of oral cancer with any variant allele at KRAS-LCS6 had significantly reduced survival [69]. MiR-375 was confirmed as a strongly repressed miRNA in OSCC. Wiklund et al. reported a 10-fold decrease of expression in tumours compared to matched adjacent tissue, and a 1000-fold change when compared to healthy epithelium [70]. They also suggested that miRNA-375 was probably epithelial specific, highlighted by two orders of magnitude higher miR-375 expression in healthy epithelium than stroma. Multivariate analyses showed miR-375 to be increased with alcohol consumption and demonstrated higher expression in tumours of pharyngeal and laryngeal origin compared with oral cavity tumours. This differential expression confirmed that miRNA profiles are a tumor- and cell-type specific and have the potential to differentiate tumour subtypes [71]. miR-375 expression was significantly associated with late stage disease, larger tumor size and the non-cohesive type of pattern of invasion in OSCC [44]. Consistent with repression in tumors miR-375 levels were lower in both patient oral rinse and saliva [70].

The first reported oncogenic miRNAs were miR-155 and the miR-17-92 cluster (Croce, 2009). MiR-155 expression in OSCC cells and tumour tissues was significantly higher than that of controls [72], [73]. It plays a tumor-promoting role in OSCC by regulating the BCL6/cyclin D2 axis [72]. Rather et al. discov-
ered ectopic expression of miR-155 in HEK293 cells dramatically reduced CDC73 levels, enhanced cell viability, and decreased apoptosis [74]. Overexpression of miR-155 led to the knockdown of CDC73 but also inverted the effect of CDC73 by promoting proliferation and suppression of apoptosis [74]. This correlates with more recent findings that miR-155 mimics enhanced CAL27 cell proliferation, migration and invasion ability, downregulated BCL6 levels, and increased cyclin D2 expression [72]. MiR-155 was overexpressed in OSCC, and it was located in the cancer nest, inflammatory area, and vascular endothelium of OSCC suggesting that high miRNA-155 expression level in cancer-free mucosal tissues may predict poor prognosis in patients with OSCC [73]. A recent study found that miR-200b and miR-155 could be potential markers for personalised treatment selection of two standard regimens of chemoradiation in patients with HNSCC [75].

MiR-99 and miR-99 family, are well-known miRNAs that define the signature and contribute to the tumorigenesis of HNSCC [76][77]. Their function in OSCC is relatively unclear. In a study by Wang et al. the expression levels of miR-99a were markedly decreased in OSCC tissues compared with the adjacent non-tumour tissues. The results of their in vitro experiments showed that miR-99a mimics significantly inhibited the proliferation of tongue squamous carcinoma cell line and that miR-99a mimics markedly induced the apoptosis of these cells stating that miR-99a regulated the growth and survival of OSCC cells and may be exploited as a biomarker and therapeutic target for patients with OSCC [78]. Wong et al. suggested a tumour suppressive role of miR-99a [46]. MiR-99a downregulation was confirmed both in tested oral cancer cell lines and clinical specimens. Its ectopic expression inhibited oral cancer cell migration and invasion while anti-miR-99a, silencing miR-99a functions, had the opposite effect [79]. Together with miR-375 and miR-1-2-3p, miR-99a showed depression in an ever smoker tumour group versus controls [67].

Recent work has brought to light two distinct etiologies of OSCC, those associated with the primary risk factor known, tobacco usage, and those not[98][99][100]. Oropharyngeal cancer, unlike OSCC, is often associated etiologically with transforming HPV, specifically HPV16 [95] [96][97].

miRNA profile of HPV-related oral cancers

Human papillomaviruses (HPVs) are small double-stranded DNA viruses that infect squamous epithelia. Viral persistence followed by HR-HPV infection, particularly HPV-16 and 18, is a crucial risk factor for carcinogenesis [83]. Besides the effect of viral oncogenes E6/7, many reports have indicated that HPV infection could change the status of the host immune system [84]. Patients with HPV-positive HNSCC tend to be younger and have a lower intake of tobacco and alcohol. Human papillomavirus-positive HNSCC appears to be different from HPV-negative HNSCC in both molecular and clinical features [85]. One of the most common tumour suppressor proteins, p53, is mutated in up to half of oral cancers but is very rarely mutated in HPV-positive NHSCC. Tumors with a high viral load have a better prognosis compared to tumours with a low viral load or tumours that are HPV-negative [86][87][88][89]. Patients with HPV-positive oral tumours have a better response to chemotherapy, radiation, and surgery, despite having frequent metastasis to regional lymph nodes [87] and have evidence of immune activation against viral antigens [90]. Moreover, HPV-positive tumours are usually smaller in T-size but with large cystic nodal involvement (N+).

According to recent studies, miR-155 expression levels were significantly higher in HPV-positive tumours compared to both HPV-negative (SCCs) and HPV-negative tissues. The only partial similarity of miRNA profiles between oral and pharyngeal cancers as well as oral and pharyngeal mucosa was found [77]. This emphasises the importance of obtaining site-specific, e.g. oral mucosa, miRNA signatures to verify the origin of a tumour, its association with risk factors for HPV infection, and thus determine more precisely its prognosis and even generate a classifier that could differentiate between healthy epithelium and different subtypes of malignant lesions. The only partial similarity of miRNA profiles between oral and pharyngeal cancers as well as oral and pharyngeal mucosa was found [77]. This emphasises the importance of obtaining site-specific, e.g. oral mucosa, miRNA signatures to verify the origin of a tumour, its association with risk factors for HPV infection, and thus determine more precisely its prognosis and even generate a classifier that could differentiate between healthy epithelium and different subtypes of malignant lesions.

Smoking and alcohol consumption effect on miRNA expression

Tobacco and alcohol are the leading environmental risk factors in the development of human diseases modulating the expression of miRNAs through various signalling pathways, such as apoptosis, angiogenesis, and inflammatory pathways [92]. Studies overall make it clear that there is much more to be done to more definitively elucidate the effects of cigarette smoke (of any type) and alcohol on non-coding RNA expression and the later downstream effects on behavioural health and other medical conditions [93].

Using RNA-sequencing data from 136 HNSCC patients obtained from The Cancer Genome Atlas (TCGA) 8 miRNAs were found to be significantly upregulated in alcohol-associated HNSCCs (Table 2). qRT-PCR experiments in vitro determined that among these candidates, miR-30a and miR-934 were the most highly upregulated miRNAs by alcohol and acetaldehyde. Overexpression of miR-30a and miR-934 in normal and HNSCC cell lines produced up to a 2-fold increase in cellular proliferation, as well as induction of the anti-apoptotic gene BCL-2. Upon inhibition of these miRNAs, HNSCC cell lines exhibited increased sensitivity to cisplatin and reduced matrigel invasion [81]. In a study with 52 OSCC tissue samples and eight independent normal tissues expression levels of miR-34a were found to be comparatively high in patients with a history of alcohol con-
consumption. They were associated with histological grade and alcohol consumption status [82]. Previous reports stated that ethanol feeding for four weeks significantly up-regulated 0.8% of known miRNAs compared with controls, including miR-34a which also altered expression of matrix metalloproteases 1 and 2 [94].

When examining miRNA levels and their association with OSCC in tobacco users - miRNAs in OSCCs of ever smokers versus that in nonmalignant lesions in a similar population, Kolokythas et al. found up-regulation of miR-196a-5p which is associated with oral cancer in many studies. They also compared miRNAs enriched with tobacco-associated OSCC versus miRNAs in healthy oral tissue and found induction of 8 miRNAs including miR196a-5p, miR-10b-5p, miR-31-5p, miR-451a and miR-144-3p. Furthermore, miRNA levels in never-smoker OSCCs were compared to that in non-pathological mucosa in never smokers and seven - miR-196a-5p, miR-10b-5p, miR-503-5p, miR-451a, miR-144-3p, miR-187-3p and miR-31-5p, showed increased expression in the OSCC samples of the never smokers. Finally, they compared OSCC miRNA expression in ever-smokers versus never smokers, and it showed similar levels - all ten miRNAs enriched in the never smoker OSCCs by rank product were contained in the list of miRNAs enriched with the ever smoker group. The direct comparison between the last two groups revealed only one miRNA was differentially expressed, miR-637 (Table 2), confirming how similar the two groups were. This finding led the authors to conclude there was no significant difference between ever and never smokers [67].

In summary, there are very few studies and not enough data gathered to delineate specific miRNA profiles of oral cancers in smokers vs non-smokers and drinkers vs non-drinkers. If OSCC in never smokers is a distinct subtype of this cancer then regulatory RNAs in such tumour tissue may be different from that of tobacco-associated OSCC. Although a study found that smoking had only a minor impact on the types of mutations observed in SCC of the oral tongue, different miRNA profile of oral cancers in smokers vs non-smokers could be expected as in investigations on malignant mesothelioma [91]. A study by Pickering et al. found that gene-specific mutation and copy number alteration frequencies were similar between young and old patients with squamous cell carcinomas of the oral tongue in two independent cohorts, stating what types of base changes observed in the young cohort were similar to those in the old cohort even though they differed in smoking history [101]. However, only one miRNA from just one study was identified as differentially expressed in these two groups of cancers [67].

Concluding remarks and future potential

miRNAs have become the focus of intensive research. There are numerous studies adding information on specific miRNAs with altered expression in oral cancer thus contributing to the construction of a particular miRNA signature of the disease. Further steps in the same direction could lead to the formation of miRNA signatures of different stages or specific subtypes of OSCC, including subtypes dependent on the presence or absence of known risk factors. Overall the effect of HPV status and presence of risk factors such as smoking and alcohol consumption has been revealed by abundant data connecting them to the pathogenesis and the development of oral and oropharyngeal SCC. However, data about specific miRNA expression in oral cancer depending on presence or absence of these risk factors is relatively scarce. The results of many analyses have shown some consistencies in microRNA differentiation, such as a universal upregulation of miR-21-5p, and somewhat lower consensus on other potential oncomirs, probably due to the variable amount of mixed epithelium/stroma in samples and diversity of aetiology of the tumour subtypes. One of the main concerns that need to be addressed especially in future studies is the type of tissue obtained by biopsy techniques – for example, scalpel or brush biopsy. Focusing on one/single or multiple cell types - epithelium, stroma, could make the studies more sensitive to changes in expression of miRNAs found mainly in this specific cell type. For example, miRNAs enriched in stroma and not epithelium of tumours would not be apparent. Some samples may also have blood contamination while others do not. In particular, malignant lesions are more highly vascularized next to epithelial cells, which can significantly increase the mix of blood cells exposed. For this reason, there must be a correct protocol for harvesting samples for miRNA examination. The intensive search for specific miRNA signatures of oral cancers associated with different risk factors continues. Each new piece of evidence will shed additional light on the process of carcinogenesis and mechanisms of OSCC development and progression.

Authors’ Statements

Competing Interests

There were no financial support or relationships between the authors and any organization or professional bodies that could pose any conflict of interests.

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