Role of Salivary microRNAs in Oral Cancer, a Review

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Abstract

Head and neck cancer (HNC) is a pressing global public health concern characterized by a challenging prognosis and a higher risk of local recurrence in cases of delayed diagnosis. Oral squamous cell carcinoma (OSCC), especially prevalent, often manifests itself initially as oral potentially malignant disorders (OPMD). Despite accessibility to the oral cavity, timely identification of OSCC remains a substantial challenge. MicroRNAs (miRNAs), a subset of small noncoding RNAs, can be released in various bodily fluids, including blood, serum, tissue, and saliva. Moreover, previous research underscores the significant role of miRNAs from oral samples as biomarkers in diverse cancers. The primary objective of this research is to discern distinct salivary miRNAs in patients with head and neck squamous cell carcinoma. This study aims to offer an overview of the fundamental attributes of saliva, biomarker detection methods in different salivary analyzes, and the potential of salivary biomarkers in the screening, monitoring, and analysis of the molecular pathology of patients afflicted by HNC. It intends to explore the prospective role of salivary biomarkers in screening, monitoring, and diagnosing cancers impacting the oral cavity and beyond.

In conclusion, miRNA sampling is a less invasive method than other methods, such as blood and tissue, for the early diagnosis of lesions with a potential to turn into cancer, especially oral cancer and other cancer diagnosis with reduced anxiety and pain for the patient. Moreover, it is much easier to obtain multiple samples at various times for screening and monitoring, especially potentially malignant disorders, and cancer.

Keywords: head and neck cancer, miRNA, oral cancer, oral squamous cell carcinoma.

Introduction

Head and neck cancers (HNC) encompass a spectrum of malignancies affecting various anatomical regions, including the lip, tongue, salivary glands, oral cavity, pharynx, nasopharynx, hypopharynx, nose, sinuses, thyroid, ear, and larynx [1]. Squamous cell carcinomas of the head and neck (HNSCC) constitute the most prevalent subtype [1].

Significant temporal increases in oropharyngeal cancers were documented in Australia from 1987 to 2006 [2]. In the United States, HNC accounts for 3% of newly diagnosed cancers and contributes to 2% of annual mortality [3]. Demographic trends suggest a higher incidence of oral and oropharyngeal cancers among females (3%) compared to males (2%) [4]. The predominant tobacco use, accounting for 75% to 90% of cases, is the main etiological factor for HNC [5]. Furthermore, alcohol consumption independently increases risk, and the synergistic effect of combined tobacco and alcohol use further increases the likelihood of HNC [6, 7]. Human papillomavirus (HPV) infection, notably associated with tonsillar and oropharyngeal squamous cell carcinomas, has contributed notably to the increased prevalence of oropharyngeal and base tongue cancers [8, 9].

Therapeutic modalities for HNC, which include radiation therapy (RT), chemotherapy, and surgical intervention, have shown limited success, with estimated prognostic outcomes ranging between 40% and 50% in recent decades, often correlated with delayed diagnosis [8]. On the contrary, the impact of the HPV vaccine on oral and oropharyngeal cancers, analogous to its effect on cervical cancers, remains under ongoing investigation [10]. Molecular marker elucidation in head and neck squamous cell carcinoma (HNSCC) lags behind comparative efforts in breast and lung cancers [11, 12]. However, the robustness of microRNAs (miRNAs) under adverse conditions, coupled with...
Efficient healthcare services have contributed significantly to complete remission for numerous patients, with a survival rate of 50% that extends beyond five years after diagnosis [21]. Survival outcomes are based on factors such as tumor location, size, and stage [22]. Advancements in surgical procedures and radiochemotherapy have notably increased patient survival rates in recent times [23].

**Epidemiology of head and neck cancer**

Head and neck cancer is among the most common malignancies worldwide, particularly prevalent in developed nations [24]. Globally, the incidence rate in males is 17.7 per 100,000 individuals and 5.3 per 100,000 in females [25]. In India, the incidence of head and neck cancer in males and females is notably high at 25% and 10%, respectively, largely attributed to increased tobacco consumption (especially chewing tobacco and betel quid) and alcohol consumption [26]. Sugerman et al. reported 2173 new oral cancer cases in Australia in 1996, with a higher incidence among males (1490 cases) compared to females (683 cases), constituting about 3% of all cancer-related deaths in Australia that year [27]. Furthermore, male dominance was observed in incidence rates, with a ratio of 4.4:1 for pharyngeal cancer and 2:1 for oral cancer compared to women [28]. Studies also indicate that lower socioeconomic status and malnutrition increase susceptibility to head and neck cancer [29].

In the United States, cancer is the second leading cause of mortality, accounting for 23% of all deaths. However, survival rates for oral cancer and larynx cancer were notably low between 1974-1976 and 1992-1998, at 3% and 5%, respectively [30]. Oral cancer holds a substantial position within head and neck cancers, with an incidence rate of 5.29 in males and 5.75 in females per 100,000 individuals. Another study revealed rates of 5 and 1.6 per 100,000 population in males and females, respectively [25]. In Canada, head and neck cancer ranks as the seventh most common cancer in males and thirteenth in females. More than 4000 new cases were estimated in 2003, predominantly affecting males at a rate of 75% [31]. The prevalence rates for oral and pharyngeal cancers in males were estimated at 12 per 100,000, while laryngeal cancer stood at 6 per 100,000, contrasting with a lower incidence in females at 5 per 100,000 for oral cavity and pharyngeal cancer [31].

Although there has not been marked improvement in overall head and neck cancer survival rates in Canada, specific regions like Quebec witnessed noteworthy progress. Between 1984 and 1998, 5-year survival rates for males with oral cavity cancer improved from 34% to 90%, for pharynx cancer from 27% to 47% and for larynx cancer from 63% to 66%. Similarly, in females, survival rates for oral cavity, pharynx, and larynx cancers varied from 26% to 98%, 23% to 67%, and 65% to 72% respectively. However, these improvements were predominantly observed among male patients with oral cavity cancer, while survival rates at specific sites remained modest, ranging from 3% to 12% [31].

**Oral Cancer**

Oral cancer manifests itself through various pathologies and etiological factors, commonly associated with alcohol consumption and tobacco use [32]. Squamous cell carcinomas (SCC) predominantly affect the lip, oral cavity, and oropharynx [33]. According to the International Classification of Diseases in Oncology, Third Edition (ICD-O-3), oral cavity cancers are classified into specific anatomical sites, including the tongue, gum, cheek, floor of the mouth, palate, and other mucosal regions [34] (refer to Table 1).
Globally, oral squamous cell carcinoma (OSCC) ranks sixth in cancer incidence, with variations in patient numbers and tumor locations in different geographical regions [35]. Regions such as South East Asia, Latin America, and eastern Europe demonstrate notably high incidence rates [35]. The main risk factors contributing to oral cancer include tobacco and alcohol consumption, along with areca nut consumption [36]. Reports highlight a rising incidence of oral cancer cases among individuals younger than 40 years [37]. In Australia, the lip emerges as the predominant site of oral cancer due to extensive exposure to ultraviolet light [35]. The elevated rates of oral cancer in Australia are attributed to heavy smoking, alcohol intake, and inadequate consumption of fruits and vegetables [38]. Despite the contribution of Human Papillomavirus (HPV) to the increase in oropharyngeal cancer cases among Australian males, a decrease in the incidence of oral cancer has been observed in recent years [39].

Oropharyngeal squamous cell carcinoma (OPSCC) poses a considerable global health burden, registering an estimated 563,000 new cases and 301,000 associated deaths in 2002 [40]. Principally, tobacco and alcohol usage stand as primary etiological factors in these malignancies [40]. Despite the decline in tobacco consumption observed in developed countries, the incidence of oropharyngeal cancers has not subsided. In particular, between 1989 and 2006, while head and neck tumor incidences decreased or remained stable in the Netherlands, a discernible increase of 3% in men and 2.3% in women in oropharyngeal cancers was observed [41]. Analogous trends of increasing OPSCC occurrences have surfaced in the United States, Sweden, and Finland [42]. Scotland, in particular, witnessed a 2.9-fold increase in male oropharyngeal cancer rates over the past two decades, significantly contributing to the country’s overall elevated cancer rates [42].

Furthermore, Australian studies indicated a more pronounced rise in OPSCC incidence among males compared to females [39]. The rise in OPSCC cases is widely attributed to the prevalence of Human Papillomavirus (HPV) [43]. Oncogenic HPV proteins (E6 and E7) exhibit substantial involvement in cervical carcinoma and anal cancer, resulting from the integration of the virus genome into the OPSCC cell genomes [42]. The risk factors associated with OPSCC demonstrate robust correlations with HPV prevalence, infection rates, and associated behavioural patterns [44].

Laryngeal cancer

Predominantly observed in many Western countries, larynx squamous cell carcinoma is the most common variant. The main risk factors associated with this type of cancer include smoking and alcohol consumption [45]. Additionally, exposure to asbestos, industrial pollutants, genetic predisposition, and suboptimal consumption of fruits and vegetables have been identified as additional contributing factors to laryngeal cancer [46]. This disease predominantly affects males above 40 years old, exhibiting notably higher mortality rates compared to females [47]. However, studies in various countries have indicated a decline in laryngeal cancer cases among males while stability has been observed among females, leading to a general decrease in the incidence of this cancer [48]. These epidemiological shifts have been linked to changes in smoking habits as a probable causal factor.

Nasopharyngeal Cancer

Nasopharyngeal carcinoma (NPC) has the second highest prevalence among cancers in Hong Kong [49]. Extensive histological investigations have delineated distinctive differences in the epidemiology, pathology, clinical presentations, treatment modalities, and prognostic outcomes of NPC compared to other malignant head and neck malignancies.

Table 1: Classification of cancer that affects the oral and head and neck region

<table>
<thead>
<tr>
<th>Site</th>
<th>ICD-O-3 code</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base of tongue, lingual tonsil</td>
<td>C019, C024</td>
<td>[39]</td>
</tr>
<tr>
<td>Tonsil, Waldeyer ring</td>
<td>C090-C099, plus C142</td>
<td></td>
</tr>
<tr>
<td>Oropharynx</td>
<td>C100-C109</td>
<td></td>
</tr>
<tr>
<td>Tongue</td>
<td>C020-C023, C028, C029</td>
<td></td>
</tr>
<tr>
<td>Gum and cheek</td>
<td>C030-C039, C060-062</td>
<td></td>
</tr>
<tr>
<td>Floor of mouth</td>
<td>C040-C049</td>
<td></td>
</tr>
<tr>
<td>Palate and other mouth</td>
<td>C050-C059, C068, C069</td>
<td></td>
</tr>
<tr>
<td>Mucosa of the lip</td>
<td>C003-C005</td>
<td></td>
</tr>
<tr>
<td>Other oral cavity sites</td>
<td>C03.0, C03.1, C03.9, C04.0, C04.1, C04.8, C04.9, C05.0, C06.0, C06.1, C06.2, C06.8, and C06.9</td>
<td>[82]</td>
</tr>
<tr>
<td>Larynx</td>
<td>C32.0, C32.1, C32.2, C32.3, C32.8 and C32.9</td>
<td></td>
</tr>
<tr>
<td>Other oropharynx sites</td>
<td>C05.1, C05.2, C05.8, C05.9, C10.0, C10.1 and C10.3</td>
<td></td>
</tr>
</tbody>
</table>

(International Classification of Diseases in Oncology, Third Edition (ICD-O-3*)

Salivary microRNAs & Oral Cancer
Epstein-Barr virus (EBV) has garnered significant attention as a potentially associated pathogen linked to NPC [50]. The peculiarities of epidemiology, which include early detection, patient surveillance, and therapeutic approaches, are key characteristics of NPC [51]. Regions like southern China and South East Asia exhibit notably elevated NPC incidence rates, contributing to 18% of all cancer cases, a trend distinct from other Asian countries [49].

Salivary gland cancer

Salivary gland cancer comprises a diverse spectrum encompassing 24 heterogeneous subtypes, making up approximately 5% of the broader category of head and neck cancers (HNC) [52]. Globally, the annual incidence of new cases is estimated to be around three per 100,000 individuals, with the United States reporting an average of 2500 new cases annually [53]. Epidemiological investigations conducted in Australia have highlighted a higher prevalence of major salivary gland cancer in males compared to females [27]. Significantly, the parotid gland demonstrates a greater susceptibility to salivary cancer, albeit with predominantly benign tumors. On the contrary, malignancies in other salivary glands, such as the submandibular, minor salivary and sublingual glands, exhibit varying proportions of benign tumors, ranging from 40% to 90%, which differs notably from the relatively lower incidence of benign tumors in the parotid gland, approximately 20 to 25% [53].

Lymphomas

Lymphoma, a heterogeneous group of malignancies originating from B and T lymphocytes within lymphoid tissue, comprises Hodgkin lymphomas (HL) and non-Hodgkin lymphomas (NHL) as its primary subtypes [54]. Recent studies have revealed the clonal B cell origin in predominant lymphocytes and those found in Hodgkin lymphocytes. However, the precise etiological mechanisms underpinning the pathogenesis of lymphoma remain incompletely understood [55, 56]. Although young adults and adolescents exhibit a modest increase in the incidence of Hodgkin lymphoma [55], a substantial finding suggests that a significant portion, approximately one third to one half, of Hodgkin lymphoma cases contain the Epstein–Barr virus (EBV) genome, even in the absence of immune deficiencies [55]. In particular, individuals with EBV-associated Hodgkin lymphoma face a higher risk of lymphoma development compared to those with infectious mononucleosis, particularly those carrying the HLA-A*01 allele [57]. However, despite the observed association, conclusively establishing EBV as the primary causative agent of Hodgkin lymphoma remains a challenge, given the significant subset of cases of Hodgkin lymphoma not related to EBV [58].

The significant potential of miRNAs as a biomolecular marker in cancer

MicroRNAs (miRNAs) play fundamental roles in intricate biological processes that encompass development, differentiation, apoptosis, and proliferation. Their resilience to adverse conditions such as extreme pH, high temperatures, and repeated freeze-thaw cycles underscores their utility in investigating cancer through various bodily fluids [59].

MiRNAs have been implicated in the pathogenesis of various diseases. Neurological conditions such as Alzheimer’s and Parkinson’s disease exhibit altered miRNA expression profiles in nervous tissues compared to other anatomical sites [60]. Dysregulated miRNA expression is significantly associated with several types of cancers, including breast, lung, colorectal, pancreas, prostate, and hematologic cancers [61]. These aberrations often modulate oncogene expression or suppress tumor suppressor genes, thus contributing to tumorigenesis [62].

Different miRNA expression profiles are discernible across different types and stages of cancer, which helps with diagnostic precision [63]. Therapeutic prospects using locked nucleic acid (LNA) modified oligonucleotides and anti-miRNA oligonucleotides hold promise for diverse medical applications [64]. MiRNA expression profiles offer information on the origins of unidentified cancerous tissues, reflecting the developmental states and differentiation of cancers [63].

MiRNA signatures, particularly found in circulating serum miRNAs, serve as significant diagnostic biomarkers for cancer. Specific miRNA species such as miR-25 and miR-223 exhibit higher expression in cancer patients compared to healthy cohorts [63]. Elevated miRNA levels in the serum of patients with ovarian cancer underscore their diagnostic potential [63]. Furthermore, specific miRNA expression patterns correlate with survival rates in acute myelogenous leukemia, indicating prognostic implications [65]. These findings underscore the clinical utility of miRNA expression profiles in cancer diagnosis and prognosis.

MiRNAs markers in different types of cancers

Differential expression patterns of microRNAs (miRNAs) in various types of cancer present promising prospects for cancer diagnostics. Prostate cancer, for example, showcases identifiable markers such as miRNA-141 in patients, while specific prostate cancer xenograft models in mice allow the detection of circulating miRNAs in blood [66]. In B-cell lymphoma, miRNA-21 in serum emerges as a valuable biomarker, and the diagnostic potential of miR-142-3p and miR-509 has been observed in lymphoma cases [66-69].

Numerous miRNAs, including miR-451 and miR-214, have been implicated in drug resistance mechanisms in breast and ovarian cancers [14]. Different miRNA expression profiles, such as elevated miR-122a and miR-200c in liver tumors or miR-500 as a marker for aggressive hepatocellular carcinoma, underscore diagnostic relevance across different types of cancer [69, 70]. In particular, specific miRNAs such as miR-17-3p and miR-92 exhibit altered plasma levels in patients with colorectal cancer before and after surgery, indicating their potential as diagnostic biomarkers [71].

The diagnosis of colorectal cancer, in particular, demonstrates a specificity with miRNA-92 in plasma, distinguishing it from gastric cancer and inflammatory conditions [67]. Differential miRNA expression patterns in various cancers, including colon, pancreatic, and stomach cancers, manifest in tissues compared to normal tissue [69]. Gastric cancer pa-
tients exhibit distinctive plasma miRNA concentrations, with altered levels of miR-17-5p, miR-21, miR-106a, miR-106b, and let-7a [86].

Pancreatic cancer cases reveal elevated miR-210 in plasma, along with increased levels of miR-21, miR-155, miR-196a, and miR-210 in pancreatic ductal adenocarcinoma [72, 73]. Head and neck cancer (HNC) reflect altered miRNA expression profiles, including miR-18a, miR-375, and miR-106b-25 upregulated clusters, while miR-203 shows downregulation in HNC tissue [74, 75, 76]. Additionally, genetic alterations in chromosomal miRNA regions are associated with head and neck squamous cell carcinoma (HNSCC) [75].

Several miRNAs exhibit regulatory relationships with P53, while transcription factors such as ZEB1 / ZEB2, influenced by miR-200, modulate Epstein-Barr virus latency in epithelial cells, potentially linking EBV to HNC [77, 78]. Methylation alterations that affect miR-137 and miR-193a expression regulate OSCC cell lines [79]. Tongue squamous cell carcinoma (SCC) cells exhibit increased expression of miRNA-184, and its inhibition restricts cell proliferation [80, 81]. These findings underscore the diagnostic potential of miRNAs in cancer. Studies exploring miRNA expression in diverse HNC samples highlight the diagnostic and prognostic utility of circulating miRNAs in cancer management (Refer to Tables 2 & 3 for comprehensive insights).

### Table 2. Identified and unique miRNAs in different types of cancers

<table>
<thead>
<tr>
<th>Cancer</th>
<th>miRNA</th>
<th>Condition</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPC</td>
<td>miR-203</td>
<td>downregulated</td>
<td>Yu, Lu [76]</td>
</tr>
<tr>
<td>nasopharyngeal carcinoma (NPC)</td>
<td>miR-18a</td>
<td>Upregulated</td>
<td>[74]</td>
</tr>
<tr>
<td>classic Hodgkin lymphoma (cHL) and reactive lymph nodes (RLNs)</td>
<td>miR-96, miR-128a, and miR-128b</td>
<td>downregulated</td>
<td>[83]</td>
</tr>
<tr>
<td>EBV latency type III and type I cell lines</td>
<td>miR-146a, 146a</td>
<td>Upregulated</td>
<td>[84]</td>
</tr>
<tr>
<td>infected lymphoma cell lines</td>
<td>miR-34a</td>
<td>Upregulated</td>
<td>[85]</td>
</tr>
<tr>
<td>large B-cell lymphoma</td>
<td>miRN21</td>
<td>Upregulated</td>
<td>[86]</td>
</tr>
<tr>
<td>Liver Cancer</td>
<td>miR- 122a &amp; miRNA 200c</td>
<td>Upregulated</td>
<td>[69]</td>
</tr>
<tr>
<td>Lymph node</td>
<td>miR-142-3p &amp; miR- 509</td>
<td>Upregulated</td>
<td>[69]</td>
</tr>
<tr>
<td>Colon Cancer</td>
<td>miR-29a and miR-let-7i</td>
<td>Upregulated</td>
<td>[69]</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>miR-214, miR-19b, and miR-let-7i</td>
<td>Upregulated</td>
<td>[69]</td>
</tr>
<tr>
<td>Stomach Cancer</td>
<td>miR-214, miR-19b, and miR-let-7i</td>
<td>Upregulated</td>
<td>[69]</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>miR-500</td>
<td>Upregulated</td>
<td>Yamamoto et al., 2015 [70]</td>
</tr>
<tr>
<td>Gastric Cancer</td>
<td>miR-17-5p, miR-21, miR-106a and miR-106b,</td>
<td>Upregulated</td>
<td>[71]</td>
</tr>
<tr>
<td>Gastric Cancer</td>
<td>miRNA let-7a</td>
<td>downregulated</td>
<td>[71]</td>
</tr>
<tr>
<td>Pancreatic Cancer</td>
<td>miR-210</td>
<td>Upregulated</td>
<td>[72]</td>
</tr>
<tr>
<td>Pancreatic ductal adenocarcinoma</td>
<td>miR-21, miR-210, miR-155, and miR-196a</td>
<td>Upregulated</td>
<td>[73]</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>miR-17-3p and miR-92</td>
<td>Upregulated</td>
<td>[71]</td>
</tr>
</tbody>
</table>
Table 3. Identified and unique miRNAs in oral cancers

<table>
<thead>
<tr>
<th>Cancer</th>
<th>miRNA</th>
<th>Condition</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral cancer</td>
<td>miR-125a &amp; miR-200a</td>
<td>downregulated</td>
<td>[87]</td>
</tr>
<tr>
<td>Oral cancer</td>
<td>miR-16, let-7b, miR-26a, miR-17, miR-19a, miR-486, miR-92a, miR-30e, miR-320b, miR-451, miR-7, miR-25, let-7a, miR-195, miR-624, miR-7703</td>
<td>Upregulated</td>
<td>[88,89]</td>
</tr>
<tr>
<td>Oral cancer</td>
<td>miR-29a, miR-223, miR-338-3p, miR-142-5p, let-7d</td>
<td>downregulated</td>
<td>[88]</td>
</tr>
<tr>
<td>Cell Carcinoma of the Tongue</td>
<td>miR-184</td>
<td>Upregulated</td>
<td>[81]</td>
</tr>
<tr>
<td>Oral Cancer</td>
<td>miR-184</td>
<td>Upregulated</td>
<td>[81]</td>
</tr>
<tr>
<td>Oral Cancer</td>
<td>miR-375 and miR-200a</td>
<td>downregulated</td>
<td>[90]</td>
</tr>
<tr>
<td>Oral Cancer</td>
<td>miR-31</td>
<td>Upregulated</td>
<td>[91,92]</td>
</tr>
</tbody>
</table>

Conclusions

In summary, substantial scientific research has revealed the potential of miRNAs as biomarkers, helping in prediction, diagnosis, and prognosis. In addition, emerging evidence suggests that targeting oncogenic miRNAs or introducing tumor suppressive miRNAs could offer a promising avenue for the development of innovative treatment approaches. However, while saliva-based biomarkers, including miRNAs, show promise, a substantial number require extensive validation. Current biomolecular markers, especially miRNAs, lack the requisite reliability for precise cancer diagnosis. Discrepancies evident in the literature highlight the need for extensive large-scale investigations. Such studies seek to establish robust and consistent results, ensuring increased sensitivity and specificity in discerning cancerous conditions, potentially establishing the foundation for routine screening modalities.

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Author Contributions

All authors participated in designing the research strategy, analytical strategy for this study, quality assessment, data interpretation and the final approval of the research has been done by all authors.

Conflict of interest: no competing interests.

References:

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