Cancer Research | Review

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.

Inconclusion, 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 pre-dominant 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

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their potential diagnostic utility in malignancies, including HN-SCC, has garnered attention [13, 14].

Although most of the research is primarily focused on the identification of biomarkers in serum and plasma, investigations of miRNA expression within alternative bodily fluids, such as saliva, have gained traction and endorsement [15]. Several profiling studies focusing on salivary miRNAs have discerned unique molecular signatures associated with the diagnosis, staging, progression, and prognosis of various human cancers, underscoring the promising prospects of miRNAs as vital biological markers [16]. This review attempts to scrutinize oral specimens and their miRNA profiles, with the aim of determining the feasibility of different miRNAs or their profiles for early-stage cancer diagnostics.

Head and Neck Cancer

Head and Neck Cancer (HNC) encompasses various types of cancer originating from squamous cells, adenocarcinomas, lymphomas, and rare variations that affect various anatomical regions within the oral cavity, pharynx, larynx, and associated neck organs (refer to Figure 1) [17]. Elwood and colleagues observed an upward trend in squamous cell oropharyngeal cancer (OPC) rates among males in Queensland since 2006 and in New Zealand since 2005, demonstrating annual percentage changes (APCs) of 10.6% and 11.9%, respectively [18]. The incidence of HNC is notably higher among individuals over 50 years old due to an aging population, resulting in a surge in elderly patients [19]. Gender plays a significant role, with HNC being three to four times more prevalent in women than men [20]. In Nordic countries, approximately 4500 individuals receive HNC diagnoses annually, accounting for 1.5% to 5% of all cancer types [21].
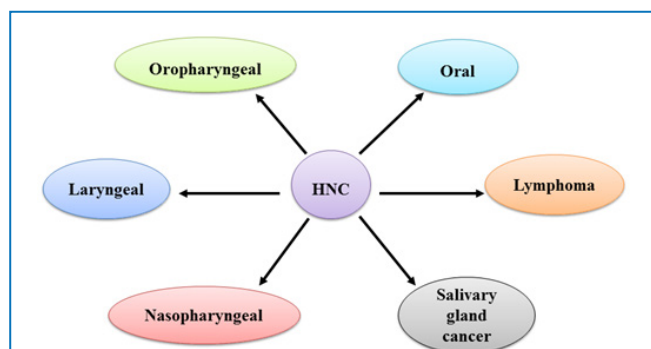
Fig.1. *Types of Head and Neck Cancer, the most common parts of head and neck cancer that affect the mouth and other items according to the International Classification of Diseases in Oncology, 3rd edition (ICD-O-3).*

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).

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

Site	*ICD-O-3 code	References
Base of tongue, lingual tonsil	C019, C024	[39]
Tonsil, Waldeyer ring	C090-C099, plus C142	
Oropharynx	C100-C109	
Tongue	C020-C023, C028, C029	
Gum and cheek	C030-C039, C060-062	
Floor of mouth	C040-C049	
Palate and other mouth	C050-C059, C068, C069	
Mucosa of the lip	C003-C005	
Other oral cavity sites	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	[82]
Larynx	C32.0, C32.1, C32.2, C32.3, C32.8 and C32.9	
Other oropharynx sites	C05.1, C05.2, C05.8, C05.9, C10.0, C10.1 and C10.3	
(International Classification of Diseases in Oncology, Third Edition (ICD-O-3*		

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 cancer

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.

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 inves-

tigating 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-106, 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

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

Table 3. Identified and unique miRNAs in oral cancers

Cancer	miRNA	Condition	References
Oral cancer	miR-125a & miR-200a	downregulated	[87]
Oral cancer	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	Upregulated	[88,89]
Oral cancer	miR-29a, miR-223, miR-338-3p, miR-142-5p, let-7d	downregulated	[88]
Cell Carcinoma of the Tongue	miR-184	Upregulated	[81]
Oral Cancer	miR-184	Upregulated	[81]
Oral Cancer	miR-375 and miR-200a	downregulated	[90]
Oral Cancer	miR-31	Upregulated	[91,92]

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.

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:

1. Gray, Henry, et al. "Gray's anatomy: the anatomical basis of clinical practice." (No Title) (2008).
2. Hong, Angela M., et al. "Squamous cell carcinoma of the oropharynx in Australian males induced by human papillomavirus vaccine targets." Vaccine 28.19 (2010): 3269-3272.
3. Jemal, Ahmedin, et al. "Cancer statistics, 2006." CA-ATLANTA- 56.2 (2006): 106.
4. Jemal, Ahmedin, et al. "Cancer statistics, 2002." Ca-A Cancer Journal for Clinicians 52.1 (2002): 23-47.
5. Walsh, Priscillla M., and Joel B. Epstein. "The oral effects of smokeless tobacco." J Can Dent Assoc 66.1 (2000).
6. Schlecht, Nicolas F., et al. "Interaction between tobacco and alcohol consumption and the risk of cancers of the upper aerodigestive tract in Brazil." American Journal of Epidemiology 150.11 (1999): 1129-1137.
7. Talamini, R., et al. "Combined effect of tobacco and alcohol on laryngeal cancer risk: a case–control study." Cancer causes & control 13 (2002): 957-964.
8. Leemans, C. René, Boudewijn JM Braakhuis, and Ruud H. Brakenhoff. "The molecular biology of head and neck cancer." Nature reviews cancer 11.1 (2011): 9-22.
9. Näsman, Anders, et al. "Incidence of human papillomavirus (HPV) positive tonsillar carcinoma in Stockholm, Sweden: an epidemic of viral-induced carcinoma?." International journal of cancer 125.2 (2009): 362-366.
10. Lowy, Douglas R., and John T. Schiller. "Reducing HPV-associated cancer globally." Cancer prevention research 5.1 (2012): 18-23.
11. Dasgupta, Santanu, et al. "Emerging strategies for the early detection and prevention of head and neck squamous cell cancer." Journal of cellular physiology 227.2 (2012): 467-473.
12. Langer, Corey J. "Exploring biomarkers in head and neck cancer." Cancer 118.16 (2012): 3882-3892.
13. Chen, Xi, et al. "Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases." Cell research 18.10 (2008): 997-1006.

14. Liu, Jeffrey, et al. "MicroRNAs, an active and versatile group in cancers." International journal of oral science 3.4 (2011): 165-175.

15. Xie, Zijun, et al. "Salivary microRNAs show potential as a non-invasive biomarker for detecting resectable pancreatic cancer." Cancer prevention research 8.2 (2015): 165-173.

16. Humeau, Marine, et al. "Salivary microRNA in pancreatic cancer patients." PloS one 10.6 (2015): e0130996.

17. Palma, Gina L. "Book Review: A Century of Progress in Head & Neck Cancer." (2016): 177-177.

18. Elwood, J. Mark, et al. "Comparison of oropharyngeal and oral cavity squamous cell cancer incidence and trends in New Zealand and Queensland, Australia." Cancer epidemiology 38.1 (2014): 16-21.

19. Syrigos, Kostas N., et al. "Head and neck cancer in the elderly: an overview on the treatment modalities." Cancer treatment reviews 35.3 (2009): 237-245.

20. Lope, Virginia, et al. "Occupation and thyroid cancer risk in Sweden." Journal of occupational and environmental medicine (2005): 948-957.

21. Parkin, D. Max, et al. "Global cancer statistics, 2002." CA: a cancer journal for clinicians 55.2 (2005): 74-108.

22. Jemal, Ahmedin, et al. "Cancer statistics, 2008." CA: a cancer journal for clinicians 58.2 (2008): 71-96.

23. Jackson, James E., et al. "Radiotherapy for perineural invasion in cutaneous head and neck carcinomas: toward a risk-adapted treatment approach." Head & Neck: Journal for the Sciences and Specialties of the Head and Neck 31.5 (2009): 604-610.

24. Parfenov, Michael, et al. "Characterization of HPV and host genome interactions in primary head and neck cancers." Proceedings of the National Academy of Sciences 111.43 (2014): 15544-15549.

25. Parkin, D. Maxwell, et al. "Estimating the world cancer burden: Globocan 2000." International journal of cancer 94.2 (2001): 153-156.

26. Yeole, Balakrishna B., et al. "Survival from head and neck cancer in Mumbai (Bombay), India." Cancer: Interdisciplinary International Journal of the American Cancer Society 89.2 (2000): 437-444.

27. Sugerman, P. B., and N. W. Savage. "Oral cancer in Australia: 1983–1996." Australian dental journal 47.1 (2002): 45-56.

28. Pisani, Paola, et al. "Estimates of the worldwide mortality from 25 cancers in 1990." International journal of cancer 83.1 (1999): 18-29.

29. Muir, Calum, and Louis Weiland. "Upper aerodigestive tract cancers." Cancer 75.S1 (1995): 147-153.

30. Jemal, Ahmedin, et al. "Cancer statistics, 2006." CA-ATLANTA- 56.2 (2006): 106.

31. Canadian Cancer Society, and National Cancer Institute of Canada. Advisory Committee on Records. Canadian cancer statistics. Canadian Cancer Society, 1987.

32. Talmi, Yoav P. "Quality of life issues in cancer of the oral cavity." The Journal of Laryngology & Otology 116.10 (2002): 785-790.

33. Ariyawardana, Anura, and Newell W. Johnson. "Trends of lip, oral cavity and oropharyngeal cancers in Australia 1982–2008: overall good news but with rising rates in the oropharynx." BMC cancer 13 (2013): 1-10.

34. World Health Organization. International classification of diseases for oncology (ICD-O). World Health Organization, 2013.

35. Warnakulasuriya, Saman. "Global epidemiology of oral and oropharyngeal cancer." Oral oncology 45.4-5 (2009): 309-316.

36. Rao, Sree Vidya Krishna, et al. "Epidemiology of oral cancer in Asia in the past decade-an update (2000-2012)." Asian Pacific journal of cancer prevention 14.10 (2013): 5567-5577.

37. Majchrzak, Ewa, et al. "Oral cavity and oropharyngeal squamous cell carcinoma in young adults: a review of the literature." Radiology and oncology 48.1 (2014): 1.

38. Bravi, F., et al. "Foods, nutrients and the risk of oral and pharyngeal cancer." British journal of cancer 109.11 (2013): 2904-2910.

39. Elwood, J. Mark, et al. "Comparison of oropharyngeal and oral cavity squamous cell cancer incidence and trends in New Zealand and Queensland, Australia." Cancer epidemiology 38.1 (2014): 16-21.

40. Parkin, D. Max, et al. "Global cancer statistics, 2002." CA: a cancer journal for clinicians 55.2 (2005): 74-108.

41. Braakhuis, Boudewijn JM, Otto Visser, and C. René Leemans. "Oral and oropharyngeal cancer in The Netherlands between 1989 and 2006: Increasing incidence, but not in young adults." Oral oncology 45.9 (2009): e85-e89.

42. Junor, Elizabeth J., Gillian R. Kerr, and David H. Brewster. "Fastest increasing cancer in Scotland, especially in men." Bmj 340 (2010).

43. Chaturvedi, Anil K., et al. "Incidence trends for human papillomavirus-related and-unrelated oral squamous cell carcinomas in the United States." Journal of clinical oncology 26.4 (2008): 612-619.

44. Smith, Elaine M., et al. "P16INK4a expression, human papillomavirus, and survival in head and neck cancer." Oral oncology 44.2 (2008): 133-142.

45. Everatt, Ruta, et al. "Consumption of alcohol and risk of cancer among men: a 30 year cohort study in Lithuania." European journal of epidemiology 28 (2013): 383-392.

46. Aune, Dagfinn, et al. "Legume intake and the risk of cancer: a multisite case–control study in Uruguay." Cancer Causes & Control 20 (2009): 1605-1615.

47. Peng, Wen-jia, et al. "Stomach cancer mortality among workers exposed to asbestos: a meta-analysis." Journal of Cancer Research and Clinical Oncology 141 (2015): 1141-1149.

48. van Dijk, Boukje AC, et al. "Progress against laryngeal cancer in The Netherlands between 1989 and 2010." International journal of cancer 134.3 (2014): 674-681.

49. Lee, Anne WM, et al. "Changing epidemiology of nasopharyngeal carcinoma in Hong Kong over a 20-year period (1980–99): an encouraging reduction in both incidence and mortality." International journal of cancer 103.5 (2003): 680-685.

50. Zhao, Fei-Peng, et al. "Levels of plasma Epstein-Barr virus DNA prior and subsequent to treatment predicts the prognosis of nasopharyngeal carcinoma." Oncology letters 10.5 (2015): 2888-2894.

51. Lo, YM Dennis, et al. "Molecular prognostication of nasopharyngeal carcinoma by quantitative analysis of circulating Epstein-Barr virus DNA." Cancer research 60.24 (2000): 6878-6881.

52. Jeannon, J-P., et al. "Management of advanced parotid cancer. A systematic review." European Journal of Surgical Oncology (EJSO) 35.9 (2009): 908-915.

53. Surakanti, Sujani G., and Mark Agulnik. "Salivary gland malignancies: the role for chemotherapy and molecular targeted agents." Seminars in oncology. Vol. 35. No. 3. WB Saunders, 2008.

54. Pace, Brian, Cassio Lynm, and Richard M. Glass. "Lymphoma." JAMA 297.18 (2007): 2044-2044.

55. Küppers, Ralf. "Clonotypic B cells in classic Hodgkin lymphoma." Blood, The Journal of the American Society of Hematol-

ogy 114.18 (2009): 3970-3971.

56. Gobbi, Paolo G., et al. "Hodgkin lymphoma." Critical reviews in oncology/hematology 85.2 (2013): 216-237.

57. Niens, Marijke, et al. "HLA-A* 02 is associated with a reduced risk and HLA-A* 01 with an increased risk of developing EBV+ Hodgkin lymphoma." Blood, The Journal of the American Society of Hematology 110.9 (2007): 3310-3315.

58. Jarrett, Ruth F. "Viruses and lymphoma/leukaemia." The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland 208.2 (2006): 176-186.

59. Schipper, Raymond G., Erika Silletti, and Monique H. Vingerhoeds. "Saliva as research material: biochemical, physico-chemical and practical aspects." Archives of oral biology 52.12 (2007): 1114-1135.

60. Kasinski, Andrea L., and Frank J. Slack. "MicroRNAs en route to the clinic: progress in validating and targeting microRNAs for cancer therapy." Nature Reviews Cancer 11.12 (2011): 849-864.

61. Esteller, Manel. "Non-coding RNAs in human disease." Nature reviews genetics 12.12 (2011): 861-874.

62. Rosenfeld, Nitzan, et al. "MicroRNAs accurately identify cancer tissue origin." Nature biotechnology 26.4 (2008): 462-469.

63. Lu, Jun, et al. "MicroRNA expression profiles classify human cancers." nature 435.7043 (2005): 834-838.

64. Yamamoto, Yusuke, et al. "MicroRNA-500 as a potential diagnostic marker for hepatocellular carcinoma." Biomarkers 14.7 (2009): 529-538.

65. Lawrie, Charles H., et al. "Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma." British journal of haematology 141.5 (2008): 672-675.

66. Ps, Mitchell. "Circulating microRNAs as stable blood-based markers for cancer detection." Proc Natl Acad Sci USA 105.30 (2008): 10513-10518.

67. Resnick, Kimberly E., et al. "The detection of differentially expressed microRNAs from the serum of ovarian cancer patients using a novel real-time PCR platform." Gynecologic oncology 112.1 (2009): 55-59.

68. Garzon, Ramiro, et al. "Distinctive microRNA signature of acute myeloid leukemia bearing cytoplasmic mutated nucleophosmin." Proceedings of the National Academy of Sciences 105.10 (2008): 3945-3950.

69. Rosenfeld, Nitzan, et al. "MicroRNAs accurately identify cancer tissue origin." Nature biotechnology 26.4 (2008): 462-469.

70. Yamamoto, Yusuke, et al. "MicroRNA-500 as a potential diagnostic marker for hepatocellular carcinoma." Biomarkers 14.7 (2009): 529-538.

71. Ayaz, Lokman, et al. "Differential expression of microRNAs in plasma of patients with laryngeal squamous cell carcinoma: potential early-detection markers for laryngeal squamous cell carcinoma." Journal of cancer research and clinical oncology 139 (2013): 1499-1506.

72. Tsujiura, M., et al. "Circulating microRNAs in plasma of patients with gastric cancers." British journal of cancer 102.7 (2010): 1174-1179.

73. Ho, Allen S., et al. "Circulating miR-210 as a novel hypoxia marker in pancreatic cancer." Translational oncology 3.2 (2010): 109-113.

74. Wang, Jin, et al. "MicroRNAs in plasma of pancreatic ductal adenocarcinoma patients as novel blood-based biomarkers of disease." Cancer prevention research 2.9 (2009): 807-813.

75. Luo, Zhaohui, et al. "miR-18a promotes malignant progression by impairing microRNA biogenesis in nasopharyngeal carcinoma." Carcinogenesis 34.2 (2013): 415-425.

76. Hui, Angela BY, et al. "Comprehensive MicroRNA profiling for head and neck squamous cell carcinomas." Clinical cancer research 16.4 (2010): 1129-1139.

77. Yu, Haibo, et al. "Epstein-Barr virus downregulates microRNA 203 through the oncoprotein latent membrane protein 1: a contribution to increased tumor incidence in epithelial cells." Journal of virology 86.6 (2012): 3088-3099.

78. Feng, Zhaohui, et al. "Tumor suppressor p53 meets microRNAs." Journal of molecular cell biology 3.1 (2011): 44-50.

79. Bartoszewska, Sylwia et al. "Regulation of the unfolded protein response by microRNAs." Cellular & molecular biology letters vol. 18,4 (2013): 555-78. doi:10.2478/s11658-013-0106-z

80. Kozaki, Ken-ichi, et al. "Exploration of tumor-suppressive microRNAs silenced by DNA hypermethylation in oral cancer." Cancer research 68.7 (2008): 2094-2105.

81. Wong, Thian-Sze, et al. "Mature miR-184 as potential oncogenic microRNA of squamous cell carcinoma of tongue." Clinical Cancer Research 14.9 (2008): 2588-2592.

82. Wong, Thian-Sze, et al. "Mature miR-184 as potential oncogenic microRNA of squamous cell carcinoma of tongue." Clinical Cancer Research 14.9 (2008): 2588-2592.

83. Hocking, J. S., et al. "Head and neck cancer in Australia between 1982 and 2005 show increasing incidence of potentially HPV-associated oropharyngeal cancers." British journal of cancer 104.5 (2011): 886-891.

84. Navarro, Alfons, et al. "MicroRNA expression profiling in classic Hodgkin lymphoma." Blood, The Journal of the American Society of Hematology 111.5 (2008): 2825-2832.

85. Cameron, Jennifer E., et al. "Epstein-Barr virus latent membrane protein 1 induces cellular MicroRNA miR-146a, a modulator of lymphocyte signaling pathways." Journal of virology 82.4 (2008): 1946-1958.

86. Forte, Eleonora, et al. "The Epstein-Barr virus (EBV)-induced tumor suppressor microRNA MiR-34a is growth promoting in EBV-infected B cells." Journal of virology 86.12 (2012): 6889-6898.

87. Lawrie, Charles H., et al. "Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma." British journal of haematology 141.5 (2008): 672-675.

88. Park, Noh Jin, et al. "Salivary microRNA: discovery, characterization, and clinical utility for oral cancer detection." Clinical Cancer Research 15.17 (2009): 5473-5477.

89. MacLellan, Sara Ann, et al. "Differential expression of mi RNAs in the serum of patients with high-risk oral lesions." Cancer medicine 1.2 (2012): 268-274.

90. Fadhil, Rushdi S., et al. "Next generation sequencing identifies novel diagnostic biomarkers for head and neck cancers." Oral Cancer 3 (2019): 69-78.

91. Wiklund, Erik D., et al. "MicroRNA alterations and associated aberrant DNA methylation patterns across multiple sample types in oral squamous cell carcinoma." PLoS one 6.11 (2011): e27840.

92. Fadhil, Rushdi S., et al. "Salivary microRNA miR-let-7a-5p and miR-3928 could be used as potential diagnostic bio-markers for head and neck squamous cell carcinoma." PLoS One 15.3 (2020): e0221779.