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Surveillance of HPV-Positive Head and Neck Squamous Cell Carcinoma with Circulating and Salivary DNA Biomarkers

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Title: **Surveillance of HPV-positive head and neck squamous cell carcinoma with circulating and salivary DNA biomarkers**

Short Title: Surveillance of HPV-HNSCC with Circulating Biomarkers

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Abstract

Head and neck squamous cell carcinoma (HNSCC) manifests in the mucosal epithelial lining of the oral cavity, oropharynx, hypopharynx, nasopharynx or larynx and has a tremendous disease burden worldwide. Smoking and alcohol consumption were once major risk factors, but HPV-associated infection has emerged as the major contributor to HNSCC occurrence in developed countries. Circulating biomarker evaluations in biofluids, also known as liquid biopsy, are an attractive alternative for cancer screening as they are minimally invasive, potentially low cost and easily repeatable on a serial basis. This review summarizes the current knowledge and potential of assessing circulating blood and salivary HPV DNA and HPV antibodies for the surveillance of HPV-positive HNSCC. Additionally, the biological underpinnings of the presence and relevance of circulating HPV DNA is discussed.

Keywords: Head and neck squamous cell carcinoma, Liquid Biopsy, circulating tumor DNA, exosomes

Abbreviations: HNSCC**,** Head and neck squamous cell carcinoma; OPSCC, Oropharyngeal squamous cell carcinoma; HPV, Human papilloma virus; AJCC, American Joint Committee on Cancer; CTC, Circulating tumour cells; ctDNA Circulating tumor DNA; cfDNA, Circulating-free DNA; NGS, Next generation sequencing; WGS, Whole genome sequencing; CAPP-Seq, Cancer personalized profiling by deep sequencing; iDES-enhanced CAPP-Seq, Integrated digital error suppression-enhanced cancer personalized profiling by deep sequencing; EFIRM, Electric field-induced release and measurement; NSCLC, Non-small cell lung carcinoma; cHPV-DNA, Circulating-HPV-DNA; MSP, Methylation-specific polymerase chain reaction; sHPV-DNA Salivary HPV-DNA; EVs, Extracellular vesicles

Introduction

HPV and oropharyngeal squamous cell carcinoma

Head and neck squamous cell carcinoma (HNSCC) develops in the mucosal epithelial lining of the oral cavity, oropharynx, hypopharynx, nasopharynx or larynx. It is estimated that HNSCC will affect more than 500,000 new cases worldwide.[1] HNSCCs are more common in men, with a peak incidence of around 60 years. Tobacco consumption and excessive alcohol intake represent the two main risk

factors for HNSCC development. Their synchronous action increases the risk of developing a malignancy by 30 times.[2] Developed countries have experienced a decrease in incidence of some HNSCC over the last few decades, potentially as the result of preventive strategies to reduce tobacco use. However, in contrast, cases of oropharyngeal squamous cell carcinoma(OPSCC) have increased in number when compared to other anatomical subsites of HNSCC.[3] The incidence of OPSCC doubled from 1990 to 2006 and again from 2006 to 2010.[4] OPSCC includes SCC of the base of tongue, palatine tonsils, soft palate and the posterior wall of the pharynx between the nasopharynx and the hypopharynx. Evidence suggests that in developed countries this increase has not been through the traditional risk factors of tobacco consumption or alcohol, but through infection with human papilloma virus (HPV).[5,6] In fact, in North America, over 56% of OPSCCs are HPV positive,[7] following by 52% in Japan, 45% in Australia, 39% in northern and western Europe, and 13% in the rest of the world.[8]

Human papilloma viruses are a heterogeneous family consisting of five phylogenetic genera (alpha, beta, gamma, mu, and nu HPVs, with at least 120 genotypes) of small non-enveloped, circular, double-stranded DNA viruses.[9] HPV targets the basal cells of genital and upper respiratory tracts and the skin stratified epithelia. The alpha genus HPV types are divided into two groups: low-risk HPVs (mainly associated with benign genital warts) and high-risk HPVs (responsible for cervical, anogenital, and oropharyngeal cancers). In the last two decades, high risk alpha HPVs, and in particular HPV type 16 (HPV16), have been causally related to a subset of OPSCC arising from the crypt epithelium of the palatine tonsils and base of tongue as well as a substantial fraction of SCCs detected in metastatic neck nodes of unknown primary.[9] HPV-positive OPSCC is now considered a rising sexually transmitted disease showing distinctive epidemiological, clinical, and molecular features. Patients with HPV-positive OPSCC are more likely to be younger, without a history of smoking and alcohol abuse, and have a higher socioeconomic and performance status than those with HPV-negative OPSCC.[10] Furthermore, HPV-positive OPSCC appears to demonstrate better

treatment outcomes compared to HPV-negative HNSCC,[11] as evidenced by the recent changes in the American Joint Committee on Cancer (AJCC) staging criteria. In HPV-positive OPSCC, p53 and Retinoblastoma pathways are both inactivated as a result of sequestration by binding viral oncoproteins. The E6 protein drives cell proliferation by stimulating ubiquitination and proteasome-dependent degradation of the p53 tumour suppressor protein.[12] E7 viral oncoprotein inhibits the Retinoblastoma pathway and furthermore, viral integration into the host genome may contribute to neoplastic transformations by deregulation of key cellular genes and induction of genome instability.[13]

OPSCC surveillance with circulating DNA biomarkers

Despite continuous research into the molecular nature of HNSCC, survival rate have only improved 3–5% in the last 2 decades. [14,15].Compared to other cancers like breast, cervical, or colorectal, HNSCC's five-year survival rate continues to range between 40% and 89% depending on primary tumor site and stage due to limited and ineffective treatment options.[16] This may be explained by the significant chance of incomplete tumour response to primary treatment (residual disease) or development of new disease after treatment has initially been successful (recurrent disease). Either of these situations have a negative impact on patient outcomes if the diagnosis is not made at an early stage. Clinical examination and imaging techniques such as computed tomography (CT), magnetic resonance imaging (MRI), or [18F]-Fludeoxyglucose–positron emission tomography[22] still have difficulties in detecting recurrence (both after surgery or chemo-radiotherapy) because of post-treatment effects on the surrounding tissue, especially during the first few months of follow-up.[18] It appears that diminished attention and funding opportunities by national granting agencies continue to limit research progression.[19] Furthermore, the diagnosis of subclinical regional or distant metastases is challenging.[20] These difficulties in surveillance and the desire for personalized anticancer therapy, has prompted investigation into circulating biomarkers as a means to improve post treatment outcomes and survival in these patients.

One of the confounding issues is that HNSCC does not have any concrete biomarkers for diagnosis like those other cancers. Currently, for HPV-associated OSCC, the emerging strategy is to utilize immunohistochemical analysis of formalin-fixed paraffin embedded biopsy samples for p16 INK4a to identify high-risk HPV in tumour tissue.[21] The biopsy sample can also be assessed for HPV DNA and RNA by polymerase chain reaction and in situ hybridization. Contrastingly, non-HPV associated HNSCC is associated with mutations in certain tumour suppressor genes such as TP53, EGFR, CDKN2A, NOTCH1, FAT1.[22] The alterations can commonly be found upon analysis of tissue biopsy samples obtained from the tumour using nasopharyngolaryngscopy, or CT/MRI guided procedures These current methods are not only invasive but lack the ability to detect early-stage disease.

Circulating biomarker evaluations in plasma and serum are attractive for cancer screening because they are blood-based tests that are minimally invasive, potentially low cost and easily repeatable on a serial basis. The development of next generation sequencing technology has allowed the concept of a 'liquid biopsy' to potentially become a reality. Potentially, the term "liquid biopsy" first emerged during a presentation at the 7th International Symposium on Minimal Residual Cancer in 2009, and a year later appeared in a formal review article suggesting that circulating tumour cells (CTC) in blood could be used for cancer detection.[23,24] Although the initial context of liquid biopsy referred to CTC detection in biofluids, it now includes circulating tumor DNA (ctDNA), circulating exosomes and other analytes in body fluids, such as serum, plasma, urine, saliva etc. Liquid biopsies have the potential to assist clinicians screen for disease, stratify patients, and follow patients undergoing surveillance. It can also offer an opportunity to detect early lesions in cases where biopsy is difficult, such as brain cancer as it has been shown that tumor DNA can be found in the cerebrospinal fluid.[25]

Cell-free ctDNA are DNA fragments, generally internucleosomal ~160 bp, thought to be derived directly from the tumour and released into the bloodstream via mechanisms of tumor cell necrosis

and/or apoptosis.[26] They contain tumor-specific genomic alterations such as mutations or methylation patterns. While normal healthy individuals have little circulating-free DNA (cfDNA) in their plasma (~30 ng/mL), a high level of cfDNA (~180 ng/mL) can be detected in the plasma of cancer patients and be used as a disease indicator.[27]

In parallel with the progression of ctDNA as a promising biomarker for cancer detection, there has a growing momentum for advancing ctDNA detection techniques. Large-scale sequencing methods such as microarray-based comparative genome hybridization, single nucleotide polymorphism analysis, and next generation sequencing (NGS) are useful for association and biomarker discovery studies but could also be applied to detect ctDNA in patient samples. Alternatively, methods like allele-specific PCR or digital PCR amplify specific sequences of DNA molecules of interest are techniques to assess ctDNA but can only target a small selection of targets in a sample. Amplicon-based NGS, whole genome sequencing (WGS) and whole exome sequencing provide comprehensive approaches to access entire genome or exome. However NGS and WGS may be too expensive or complicated, and lack adequate sensitivity to be used clinically.[28] To alleviate these concerns, cancer personalized profiling by deep sequencing (CAPP-Seq) and integrated digital error suppression-enhanced cancer personalized profiling by deep sequencing (iDES-enhanced CAPP-Seq) were developed by integrating a capture-probe to ctDNA targets of interest to deep sequencing to improve specificity.[29] Another approach, electric field-induced release and measurement (EFIRM), allows direct measurement of ctDNA in only 40ul plasma or saliva without sample processing or amplification of the clinical sample. EFIRM demonstrated exceptional performance in detecting *EGFR* ctDNAs in plasma and saliva of non-small cell lung carcinoma (NSCLC) patients.[30,31] These aforementioned techniques are all viable options for HPV ctDNA detection.

With the increasing incidence of HPV-positive OPSCC it is paramount that surveillance methods will be developed to improve outcomes in this group of younger patients.

The focus of this review is to examine the clinical potential of circulating viral and somatic ctDNA biomarkers and liquid biopsy in the surveillance of HPV-positive HNSCC. Focus will be placed on both circulating blood and salivary DNA pertinent to HSNCC detection with comment on HPV antibodies as an adjunctive circulating biomarker. Additionally, biological principles governing the presence of these clinically-viable biomarkers will be discussed.

Cell-free circulating tumour HPV DNA in serum and plasma for surveillance

In HPV-negative HNSCC, ctDNA has been investigated to reveal tumour-specific driver mutations, in addition to epigenetic modifications. For HPV-positive HNSCC, the viral genome can be detected by sequencing the cell-free DNA, negating the need to either perform whole exome sequencing of the tumour, or design complex panels for ctDNA targeted analysis to identify driver mutations. Evidence already exists that circulating EBV DNA can predict recurrence in nasopharyngeal carcinoma.[32] As such, circulating-HPV-DNA (cHPV-DNA) is a potential target for post-treatment disease monitoring in cases of HPV-positive HNSCC.[33] Research is currently focusing on cHPV-DNA as a biomarker in surveillance either as an immediate post treatment assessment of residual disease, prediction of recurrence and survival, or using serial cHPV-DNA measurements to assess treatment response.

Cao et al., using E6/E7 qPCR, detected cHPV-DNA in 65% of pretreatment plasma samples from HPV-positive OPSCC patients.[34] None of the HPV-negative HNSCC or non-cancer controls had detectable cHPV-DNA. Serial measurements in 14 patients showed rapid decline in cHPV-DNA that became undetectable upon radiotherapy completion. In 3 patients, cHPV-DNA rose to a discernable level at the time of metastasis suggesting plasma cHPV-DNA may be a valuable tool for identifying relapse. Ahn et al. analyzed pre and post-treatment HPV-DNA status in a retrospective cohort of 81 patients with HPV-positive tumours and a control group of 12 HPV-negative tumours.[35] This study performed liquid biopsy assessment from plasma and saliva, collecting post-treatment samples at 3

monthly intervals. Post-treatment HPV-DNA status was correlated with clinical outcomes, including recurrence-free survival and overall survival. Of the 93 patients, 83 had saliva samples, 61 had plasma samples and 54 had both pre and post treatment saliva and plasma samples. The sensitivity of detecting cHPV-DNA in the pretreatment plasma of HPV-positive tumours was 67.3% (35/52). Of this 67.3% with cHPV-DNA positive pretreatment plasma samples, 5 have detectable cHPV-DNA in post-treatment plasma samples, and 4 of these went on to develop recurrence with a follow-up time range of 16.0 to 60.5 months. Of the 30 patients who did not have cHPV-DNA detectable in post-treatment plasma, only 2 developed recurrence during a 3.4- to 121.0-month follow-up period. Post-treatment detectable cHPV-DNA was associated with a significantly worse recurrence free survival. Adjusting for alcohol, smoking, and T and N status, there is a significant increased risk of disease recurrence associated with cHPV-DNA positivity in post-treatment plasma samples. Surveillance of plasma HPV status had a sensitivity and specificity to predict recurrence within 3 years of 55.1% and 95.6% respectively. Plasma HPV-DNA status was not significantly associated with overall survival. Wang et al. investigated the detection of somatic mutations and the presence of HPV DNA in plasma and saliva of 93 patients with HNSCC. All 93 patients donated saliva with 47 also donating blood (plasma) samples. HPV DNA was detectable in the plasma of 86% (n=21) of the HPV-positive HNSCC patients (of which all but one was OPSCC) while only 40% (n=30) of the saliva from the HNSCC positive group had detectable cHPV DNA. This discrepancy was rationalized given the anatomical location of OPSCC; where in the same study 100% of oral cavity cancers demonstrated tumour somatic mutation ctDNA in saliva samples. The same paper also briefly reported on post treatment follow-up data for 9 patients. Although they do not explicitly state HPV status in this group, as the focus of this review is surveillance, their findings are noteworthy. Three of the nine patients had detectable ctDNA post treatment and their detection preceded clinical signs of recurrence in 2 patients by 9 and 15 months respectively.[36]

Lee et al. investigated cHPV-DNA status in plasma of 47 HPV-positive patients undergoing radical chemo-radiotherapy using an amplicon based NGS assay for detection of cHPV-DNA. This assay demonstrated sensitivity and specificity of 96% for cHPV-DNA detection pre-treatment. Forty-six of the 47 patients (97%) had PET-CT confirmed complete response, all were negative for plasma cHPV-DNA post treatment. The one patient who had a positive post treatment cHPV-DNA had residual cancer on PET-CT and biopsy.[37] Rutkowski et al. investigated cHPV-DNA status in 55 patients after radiotherapy +/- chemotherapy showing that persistent cHPV-DNA in the post-treatment surveillance phase (13 patients) predicted both local and regional failure. Although 1 patient with negative cHPV-DNA still developed recurrence and 9 patients with persistent cHPV-DNA developed no recurrence, demonstrating a mixed sensitivity and specificity.[38]

An alternative approach to target specific driver mutations in ctDNA (i.e. HPV DNA) is to analyze epigenetic alterations (e.g. hypermethylation) of specific gene sites. Nakahara et al. examined p16 methylation using a methylation-specific polymerase chain reaction (MSP) in tumour and serum samples of 17 OSCC patients.[39] Aberrant methylation was detected in 11 (64.7%) of the tumour tissues. 6 of these 11 patients (54.5%) also showed the same p16 methylation alterations in their serum. Of note is that ctDNA (p16 hypermethylation) was detected in the serum of 3 out of 4 patients with recurrence, suggesting that MSP may be a sensitive and useful method for detecting recurrent OSCC. No methylation was found in the control serum cell-free DNA.

Cell-free salivary HPV DNA

Circulating tumor DNA have also been detected in saliva. Rationale for their presence in saliva include vesicular transport, ultra-filtration from blood, passive diffusion or active transport. In addition, due to its anatomical location, saliva can also contain molecular biomarkers derived directly from the oral cancer through sloughing and necrosis of apically located tumor cells.[40] Saliva can be

easily collected repeatedly, inexpensively and non-invasively leading to a growing interest to use this bodily fluid for liquid biopsy.[30]

In 2008, Chuang et al. demonstrated the feasibility of using post-treatment salivary HPV-DNA (sHPV-DNA) as a prognostic marker for persistent or recurrent HNSCC.[40] HPV-16 presence in follow-up salivary rinses preceded clinical detection of disease recurrence by an average of 3.5 months. Patients with the presence of HPV-16 DNA in surveillance salivary rinses were at a significant risk for recurrence. Even when analyzing a relatively small number of cases, (4 of 20 HPV tumor positive patients ultimately developed recurrence, and 2 of these 4 patients were HPV-16 positive) and despite a low sensitivity (50%), the study reported no false negative results (specificity =100%): all of the patients who did not recur remained salivary HPV-negative in their surveillance.

As previously discussed, Ahn et al. reported results from 93 OPSCC patients (81 HPV positive, 12 HPV negative) with pre and post treatment plasma or saliva samples.[35] In the pretreatment phase, sHPV16-DNA had a sensitivity of 53% to predict tumour HPV status. Four of these 38 patients that had post-treatment sHPV-DNA, 3 of which developed recurrence (follow-up time range 17 to 53 months). The remaining 34 patients with HPV–positive pretreatment saliva had HPV–negative post-treatment saliva samples, but 2 of these patients developed recurrence (follow-up range 3.4 to 111.0 months). Therefore, the sensitivity and specificity of post-treatment salivary HPV status in HPV-positive tumors in predicting recurrence within 3 years were 18.8% and 96.6%, respectively. When adjusting for alcohol, smoking, T and N status, risk of recurrence was significantly increased with positive sHPV-DNA status. The authors also noted that a combination of data from pretreatment plasma and salivary samples can increase the sensitivity of pretreatment HPV status as a screening tool for HPV-positive OPSCC compared with using either sample alone. Furthermore, when combining post-treatment sHPV-DNA status with cHPV-DNA status, patients with HPV-positive

status in either plasma or saliva had significantly worse recurrence free survival and overall survival, with the sensitivity to predict recurrence rising to 69.5%.

Rettig et al. reported results from salivary samples in 124 patients with OPSCC (detection of HPV16 DNA in oral rinses was present in 54% of participants at diagnosis).[42] Oral rinse samples were collected at diagnosis and post treatment (9, 12, 18 and 24 months after diagnosis) and assayed for HPV DNA. Only 5% (6/124) had detectable HPV16-DNA in any post-treatment oral rinse (4% prevalence (4 of 113) at 9 to 12 months and 3% (3 of 89) at 18 to 24 months). Ninety three percent (62/67) of sHPV-DNA detected at diagnosis had cleared after treatment. Persistent HPV-DNA detection in oral rinses (both at diagnosis and any time after treatment) was associated with a greater than 20-fold increased risk of recurrence (hazard ratio [HR], 29.7 [95% CI, 9.0-98.2]) and death (HR, 23.5 [95% CI, 4.7-116.9]). Of note, there were also 14 new type-specific high risk-HPV infections detected after treatment in 12 patients. Thus, the majority of times that non-HPV16 high risk-HPV DNA was detected after treatment, this appeared to represent new, as opposed to persistent, high risk-HPV infections (14 of 21 type-specific infections [67%]).

HPV antibody status

Although not a circulating DNA biomarker, we discuss HPV antibody status as it appears to be a sensitive circulating biomarker, linked indirectly to cHPV-DNA. Shroeder et al. investigated HPV antibody status in 46 patients with unknown primary tumours (33 HPV-positive, 13 HPV-negative).[43] They described both a high sensitivity and specificity of HPV seropositivity for HPV-positive unknown primary SCC. They validated an algorithm to define HPV16-seropositive patients by (i) high HPV16 E6 antibody levels, or (ii) antibodies against at least three early proteins. This algorithm had shown a sensitivity of 97% and specificity of 98% for HPV16-postive OPSCC. In a limited number of follow-up serum samples from HPV-seropositive patients, a decrease in antibody levels within one year after treatment was observed even if patients remained HPV-seropositive during

follow-up. Of note, one patient with recurrence had increasing antibody levels after the initial post-treatment phase, potentially predicting recurrence.

In a large study[44] of 115 HPV-positive OPSCC patients (ascertained by p16 immunohistochemistry and/or in-situ hybridization) where blood were collected at diagnosis. 64 of these patients also had blood collected for post-treatment follow-up visits for up to two years. Antibodies to HPV16 oncoproteins L1, E1, E2, E4, E6, and E7 were assayed and they were similar to Shroeder's study with HPV16 antibody levels tending to decrease slowly over time in the post treatment phase and almost never becoming seronegative. The authors conclude that the data did not fully support the utility of HPV16 antibodies in the short-term monitoring for disease recurrence (2 years) affirming that longer-term studies would be needed to evaluate if this marker might have predictive utility for recurrence within a longer interval.

Biological Explanations of Biomarkers in Biofluids

Although there is promising evidence that cell-free biomarker targets such as cHPV-DNA or miRNA can be detected in the plasma or serum of HPV-positive HNSCC patients, the biological rationale and pathway underpinning the presence of these molecular targets is not fully understood. One potential route is through packaging and transportation in extracellular vesicles (EVs) which include exosomes, microvesicles and apoptotic bodies.[45] EVs contain protein and genetic material mirroring their cell and tissue sources.[44] Traditionally, EVs are isolated by ultra-centrifugation.[46] Additional emerging technologies and newer isolation methods have arisen such as polymer-assisted precipitation,[47] immunoaffinity-based capture beads,[48] immunoaffinity-based microfluidic chips,[49] and acoustic fluidic chips.[50]

Every size category of EVs may be pathologically relevant. Apoptotic bodies represent the final stages of the apoptotic process and have been shown to contain fragments of nucleus with traces of

ctDNA.[51,52] Experiments have hinted that apoptotic bodies of Burkitt-derived lymphocytes containing EBV DNA and DNA from apoptotic rat fibrosarcoma cells expressing H-ras^{V12} have the capability to transfer viral sequences to endothelial cells and fibroblasts if co-cultured together.[53,54] These apoptotic studies may not be directly HPV-related, but they justify interest in assessing EVs in HPV-positive HNSCC patients. Alternatively, the cHPV-DNA sequences detected in the biofluids of studies discussed earlier may be in part from apoptotic bodies. Exosomes, which are EVs that range from 30-100nm, are of particular interest, as they are involved in cell-to-cell communication.[55] Isolated exosomes from hepatitis C virus infected cells contain full length-viral RNA capable of delivering viral RNA to non-infected cells and establish an active infection.[56] This may be an alternative method of viral infection which allows the virus to avoid immune intervention. The majority of reports of communicative and regulation functions of exosomes appear associated with RNA and protein. For example, analysis of the protein composition of isolated exosomes from EBV and Kaposi sarcoma-associated herpesvirus (KSHV)-infected B cells demonstrated that out of the 233 specific proteins, 93(39%) and 22(9.4%) were specifically viral-related. Recently, however, it was discovered that exosomes contain mitochondrial and chromosomal DNA ranging from 100bp to 17 kbp.[57] Speculatively, HPV DNA sequences or HPV-related HNSCC mutated DNA sequences could be packaged and communicated in exosomes shed by HPV positive HNSCC.

The translation and production of viral E6 and E7 proteins are essential to the maintenance and progression of the cancerous phenotype in HPV-associated HNSCC.[58] Interestingly, it was identified that exosomes released by HeLa cells (derived from HPV18-infected cervix adenocarcinoma) are enriched in the survivin prooncogenic protein which may be regulated by E6/E7 expression.[59] Interestingly, experimentally in HeLa cells, siRNA downregulation of E6/E7 expression was associated with lower survivin expression in whole cell extracts but the relative level of surviving remained higher in exosomal fractions.[60] This suggests that exosomes released by HeLa may represent a blunted response to E6/E7 regulation and can continue to send potent anti-apoptotic

messages to target cells. The in-vitro experimental studies mentioned signal to the possible activity of EVs but do not reflect physiological conditions. Regardless, apoptotic bodies and exosomes may explain the presence of biomarkers related to HPV-associated cancer cells and justify their targeted isolation and detection in liquid biopsy (Figure 1).

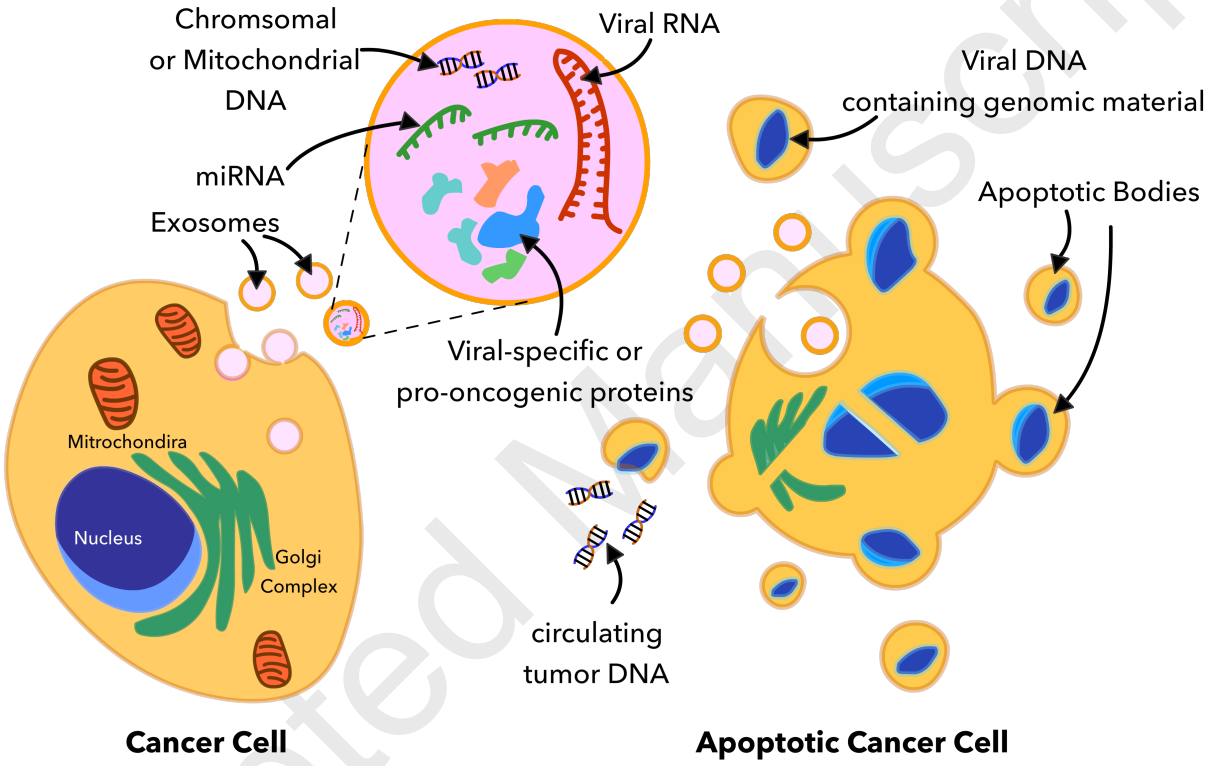

Figure 1. Potential biological sources of circulating biomarker targets in plasma/serum and saliva of HNSCC patients. Cancer cells secrete exosomes containing miRNA, viral RNA/DNA, genomic DNA, viral-specific or pro-oncogenic proteins (e.g. survivin). Cancer cells undergoing apoptosis dissemble into apoptotic bodies containing genomic material which include embedded viral DNA sequences. Ruptured apoptotic bodies or the apoptosis process itself may release DNA into the circulation (ctDNA).

Discussion

Current surveillance strategies in HNSCC are inherently inadequate. Monitoring cancer recurrence or progression requires serial testing and the need for multiple sampling by invasive tissue biopsy is

often difficult or impossible.[61] Imaging often lacks the sensitivity and specificity to detect residual disease,[62] as does clinical assessment. While evidence is limited, the ability to use circulating and salivary HPV-DNA as a biomarker of disease recurrence in HPV-positive HNSCC is now becoming a viable strategy. Indeed, this process is made simpler and more cost effective as virally induced cancers lend themselves to quantitative PCR/ targeted sequencing because of the known viral genome and oncoprotein targets.

Liquid biopsy for circulating DNA biomarkers in HPV-positive HNSCC is arguably more valuable in the post-treatment phase than for pretreatment assessment of HPV status, already achievable through tissue biopsy. It would represent a personalized strategy for surveillance, decreasing the burden of routine clinical assessment and pre-determined imaging protocols. The presence of post-treatment cHPV-DNA and sHPV-DNA indicate both an increased risk of recurrence and also poorer overall survival. The addition of HPV antibody status could be a useful adjunct in HPV-positive cancer serial surveillance. As sequencing techniques and technologies improve, the objective will be 100% concordance detection (sensitivity) to provide the ultimate complementation to the tumor-specific fingerprint (specificity) for unambiguous detection of tumor or recurrence in a noninvasive setting, both in saliva[30] and plasma.[37]

As shown in this review, combining findings from plasma and saliva samples to determine tumour HPV-status and predict recurrence in the surveillance phase can further increase sensitivity and specificity. A summary of potential targets to survey are presented (Figure 2). The future of clinical care will likely be multi-tissue/biofluid compartment liquid biopsies, that combine in a personalized patient nomogram to provide quantitative surveillance of both HPV-positive and negative HNSCC. However, it remains unknown which liquid biopsy will be of more value with regard HPV-positive HNSCC. Large cohort studies are required to demonstrate the efficacy of plasma and salivary liquid biopsies as reliable biomarkers to guide patient treatment.

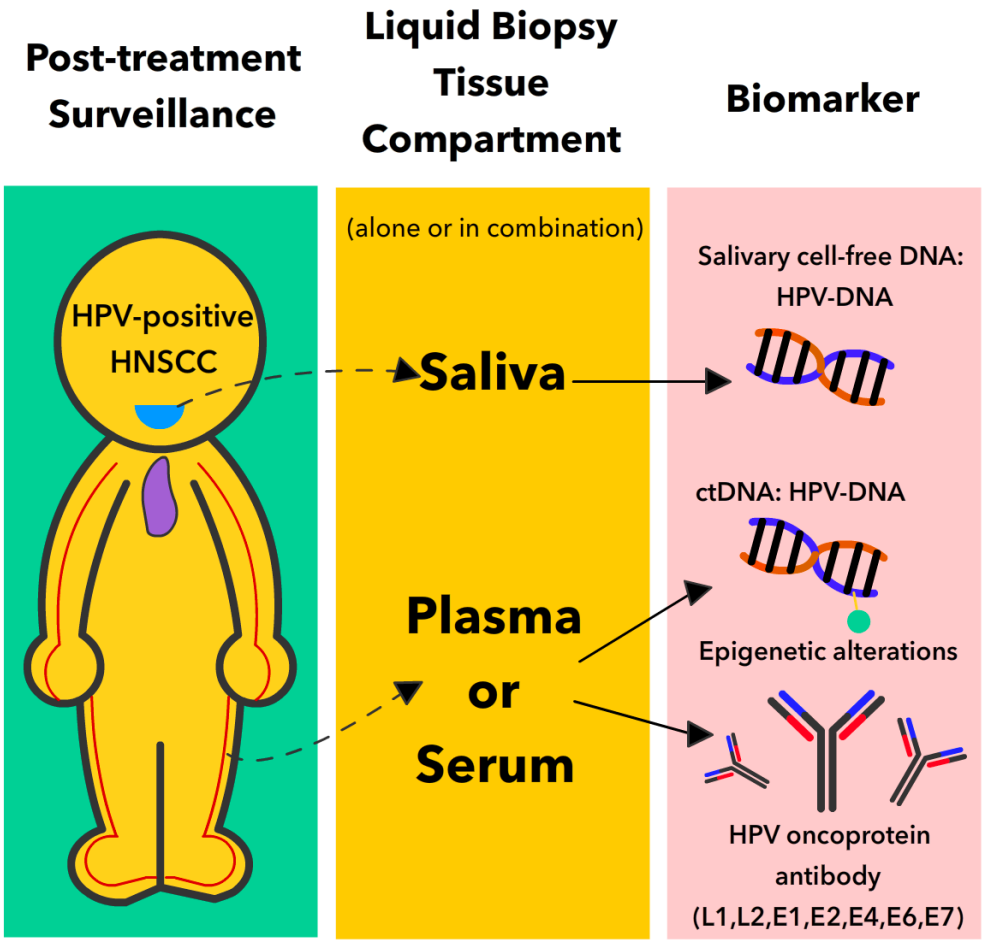

Figure 2. Schematic diagram of HPV biomarker targets and biofluid compartments for liquid biopsy surveillance of patients with HPV-associated HNSCC.

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