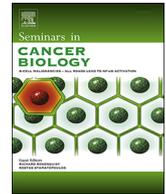




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Review

The role of tumor DNA as a diagnostic tool for head and neck squamous cell carcinoma

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ABSTRACT

Head and neck squamous cell carcinoma (HNSCC) represents the most common type of head and neck cancer worldwide. However, despite advances in cancer care globally there has been little progress in HNSCC, with survival remaining static and slightly worse in laryngeal squamous cell carcinoma with 5 year survivals remaining at ~50%. Conventional analysis of tissue through cytopathology or histopathology are the mainstay of diagnosis. Furthermore there are no useful biomarkers for disease diagnosis or surveillance. With recent technological advances, particularly in next generation sequencing, here we explore the application of tumor DNA for HNSCC diagnosis and surveillance, to improve surgical margin analysis and the potential use of molecular agents aiding in the imaging of HNSCC.

1. Introduction

Head and neck cancer is the sixth most common cancer worldwide, with more than 600,000 new cases diagnosed annually. The most common type of cancer is head and neck squamous cell carcinoma (HNSCC), representing 95% of all head and neck cancers. The majority of patients present with loco-regionally advanced disease with approximately 50% five-year overall survival rate. Even with early stage disease local recurrences occur in up to 40% of patients. Patients with recurrent or metastatic disease have a dismal prognosis of overall survival of approximately six months.

Treatment involves surgical resection, radiotherapy and/or chemotherapy. Therapy for early stage disease usually involves a single modality of either surgical resection or radiotherapy. Treatment of late stage disease involves a combination of modalities. Despite advances in diagnostics, treatment and surveillance of disease, the overall survival of patients has not significantly changed over the past 30 years. In fact, the overall survival of patients with laryngeal squamous cell carcinoma (SCC) has declined. In contrast, recently with the identification of human papilloma virus (HPV) in relation to oropharyngeal SCC, an improved prognosis has been noted in this patient subgroup when compared to HPV negative oropharyngeal SCC [1].

Recent advancements in the understanding of the molecular and genetic alterations of HNSCC following the improvement in research

techniques and bioinformatics have allowed significant strides in the understanding of the pathogenesis of HNSCC. Combined with a clear need to develop improved biomarkers for the diagnosis of HNSCC. These insights have allowed the development of molecular techniques that will aid in the screening, diagnosis and management of HNSCC that will ultimately translate to improved patient outcomes. Here in this review we will explore in particular, the applications of tumor DNA in the diagnosis of HNSCC that is demonstrated in Fig. 1.

2. Somatic mutations of head and neck squamous cell carcinomas

To understand the applications of molecular techniques in the diagnosis of HNSCC it is important to understand the cancer genomics of HNSCC as the disease involves the alteration of multiple genes and pathways, both tumor-suppressor genes and oncogenes. Below are the more commonly mutated genes and pathways.

2.1. TP53

TP53 is an important tumor-suppressor gene on chromosome 17p12, frequently inherited in Li Fraumeni syndrome [2,3], and is the most commonly mutated gene in HNSCC, with approximately 50–70% all HNSCC tumors having a TP53 mutation [4–8]. In normal cells, TP53 plays a critical role in regulating the cell cycle in response to DNA

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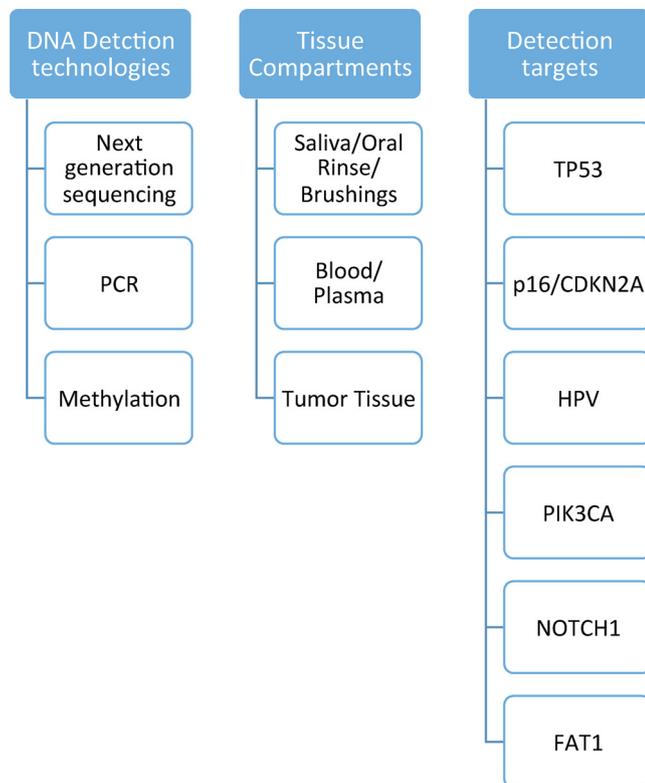


Fig. 1. Classifications of tumor DNA detection in head and neck squamous cell carcinoma by detection technologies, tissue compartments and potential targets for tumor DNA molecular diagnostics.

damage. TP53 is activated by exposure to cellular stress such as DNA damage, which results in the accumulation of active TP53 protein in the nucleus. Then transcriptional induction of downstream signaling pathways by binding to a gene's promoter and upregulates transcription of mRNA that induces cell growth arrest or apoptosis. Therefore, this growth inhibitory effect of TP53 is vital in preventing the proliferation of cells harbouring damaged DNA or of cells with the potential for neoplastic transformation [9]. In HNSCC, alterations in TP53 occur early in the premalignant squamous epithelium, before invasive transformation. With histological progression from severe dysplasia to invasive carcinoma there is an increase in TP53 mutations [10–12]. Furthermore, the frequency of TP53 genetic alterations in patients with a history of tobacco and alcohol use is almost double that of those without such history [13,14].

The importance of p53 mutations in prognosis is highlighted by a study of 560 patients with HNSCC. These patients were treated surgically with curative intent and tumor specimens were analyzed for p53 mutations, with mutations classed as disruptive and non-disruptive. The presence of any type of p53 mutation was associated with reduced survival but the association was stronger in those with disruptive mutations, highlighting the worse prognosis of HNSCC in the presence of p53 mutations [15].

2.2. CDKN2A/P16

Cyclin-dependent kinase inhibitor 2A (CDKN2A) located on chromosome 9p21 is a known tumor-suppressor gene frequently disrupted in HNSCC. It is inactivated through deletion, point mutations, and epigenetic promoter methylation. CDKN2A mutations are identified in 9–22% of all tumors [5–7]. When considering genetic and epigenetic alterations, p16 inactivation has been detected in as much as 80% of HNSCC [16]. The CDKN2A gene encodes the protein product p16 that plays an important role in regulating the G1 phase of the cell cycle. The

p16 protein binds to cyclin-dependent kinase 4 (CDK4) and CDK6, inhibiting their association with cyclin D1. This inhibition of cyclin D1/CDK4/6 complex activity prevents Rb phosphorylation and the release of E2F transcription factor, leading to the inhibition of the G1-to-S phase transition and leading to cell senescence [17,18]. Therefore, any genetic abnormalities inactivating the p16 pathway may confer growth advantages in cells and the development of carcinoma.

2.3. FAT1

FAT atypical cadherin 1 (FAT1) is one of the newest genes implicated in HNSCC that is involved in cell cycle regulation and proliferation [19–22]. Historically, the function of FAT1, located on chromosome 4q35.2, was incompletely understood, with some reports tentatively describing it as a tumor suppressor gene [19,22]. More recently, there has been a renewed interest in FAT1 after a study demonstrated that FAT1 is mutated in multiple malignancies, including glioblastoma, colorectal cancer, and HNSCC, and that FAT1 loss-of-function mutations results in Wnt pathway activation and tumorigenesis [23]. Several recently published whole-exome sequencing studies of HNSCC, identified FAT1 mutations in 12–23% of HNSCC patients [6,7]. Investigations are underway to determine if FAT1 mutation status is associated with patient outcomes and to investigate if the association between FAT1 and Wnt/ β -catenin signaling pathway represents a new therapeutic approach in HNSCC [24,25].

2.4. NOTCH

NOTCH1 was the most recent new cancer gene implicated in HNSCC development. With a mutation frequency of 14–19%, NOTCH1 is one of the most frequently mutated genes in HNSCC [5–7]. NOTCH1 gene is large and comprises 34 coding exons and encodes a transmembrane receptor that functions in regulating normal cell differentiation, lineage commitment, and embryonic development. Functionally, NOTCH1 signaling has both oncogenic and tumor-suppressive roles depending on the cellular context. For instance, activating truncation mutations in NOTCH1 have been identified in acute lymphoblastic leukemia and chronic lymphocytic leukemia, implicating NOTCH1 as an oncogene [26,27]. In contrast, the initial findings of inactivating mutations in HNSCC and the observation that loss of NOTCH1 in murine models led to skin carcinogenesis indicated that NOTCH1 may also act as a tumor-suppressor gene [28,29]. The data thus far are conflicting about the exact role of NOTCH1 in HNSCC. Most NOTCH1 mutations observed in HNSCC affect the epidermal growth factor (EGF)-like ligand-binding domain and are thought to lead to loss of function, suggesting the role of NOTCH1 as a tumor suppressor [5]. Contrary to the genetic evidence, there is evidence that NOTCH1 protein levels are elevated in HNSCC, and tumors expressing higher levels of NOTCH1 protein are associated with reduced survival as well as chemoresistance suggesting that activated NOTCH1 could function as an oncogene [30–33]. However, additional functional studies in vitro and in vivo are required to elucidate the exact role of NOTCH1 in HNSCC.

2.5. RAS

The RAS gene family consists of three genes that function as small GTPase molecules: KRAS, HRAS, and NRAS. The RAS genes play a critical role in cell signaling as part of the RAS–RAF–MEK–MAPK pathway. This pathway is involved in the regulation of cell proliferation, differentiation, morphology, and survival. The RAS gene family mutations have been implicated in approximately one-quarter of all human cancers, with KRAS being the most common and HRAS the least common [34]. However, in HNSCC, KRAS mutations are virtually absent while HRAS mutations have been described at a low frequency of approximately 4–5% [5–7].

2.6. PIK3CA

The PI3K–PTEN–AKT pathway is another critical pathway in HNSCC carcinogenesis. The PIK3CA gene is located on chromosome 3q26 and functions to convert phosphatidylinositol (4,5) biphosphate (P4,5P2) into phosphatidylinositol (3,4,5) triphosphate (PIP3), in turn activating Akt/PKB kinases. This results in the promotion of cell growth, survival, and cytoskeleton reorganization [35]. PIK3A is downstream of receptor tyrosine kinases such as EGFR, Met, and vascular endothelial growth factor receptor (VEGFR), which are known oncogenes in HNSCC. The prevalence of PIK3CA mutations was estimated to be approximately 6–21% in whole-exome sequencing HNSCC studies with increased frequency in HPV-associated HNSCC [5–7]. The overactivation of this pathway occurs through both amplification and mutations in PIK3CA as well as through PTEN loss [35]. PTEN is a key regulator of PI3K function. PTEN reverses the action of PI3K by removing the 3' phosphate and thus preventing the activation of downstream molecules such as Akt [36].

2.7. EGFR

Malignant transformation of HNSCC is also driven by alterations in growth factor signaling pathways. Epidermal growth factor receptor (EGFR), also known as HER1 or ErbB-1, is a tyrosine kinase receptor that is highly expressed in normal epithelial cells. EGFR is activated by several ligands, which induces receptor dimerization and autophosphorylation, resulting in activation of downstream signaling pathways [37]. These downstream pathways include MAPK, PI3K/AKT, ERK, and JAK/STAT genes that are critical for the regulation of cellular proliferation, apoptosis, angiogenesis, migration, and invasion [38]. The EGFR gene is overexpressed in 80–90% of HNSCC via gene amplification and transcriptional activation and correlates with worsened disease free survival and overall survival [39–42]. Clinical trials of the monoclonal antibody to EGFR - cetuximab have demonstrated the role these signaling pathways play in the treatment of head and neck cancer with significantly improved overall survival in locally advanced and recurrent or metastatic HNSCC [43,44]. In addition to overexpression, a mutant form of EGFR known as EGFRvIII has been implicated in resistance to anti-EGFR-targeted therapies [45]. This mutant form is characterized by a deletion in exons 2–7, leading to a truncated ligand-binding domain, rendering it constitutively active. Over-activation of EGFR signaling via overexpression or activating mutations enables cells to take on a malignant phenotype.

2.8. Human papillomavirus

HPV is a non-enveloped small double-stranded, circular DNA virus that infects epithelial cells [46]. Most HPV subtypes cause epithelial lesions with low malignant potential, such as warts or papillomas. However, there is a subset of high-risk HPV that leads to precancerous lesions. The molecular mechanism behind HPV-driven carcinogenesis has been extensively studied in cervical cancer. More recently, over the past decade the role of HPV in the carcinogenesis of HNSCC has also begun to be appreciated. The integration of high-risk HPV DNA into the host genome results in the expression of two key oncogenes E6 and E7 in the host cell. The E6 oncogene binds to tumor suppressor TP53, which causes the degradation of TP53 via ubiquitin-mediated processes. The degradation of TP53 prevents the host cell from engaging in cell cycle checkpoints and enduring an apoptotic response [47]. The E7 oncogene is the most important driver of cell cycle deregulation through the binding and destabilizing of the tumor suppressor retinoblastoma (pRb). This binding of pRb results in the release of E2F transcription factors, leading to the transcription of genes involved in proliferation and cell cycle progression [48]. One of the main molecular pathways amplified through E7 is the CDKN2A/p16 gene pathway, which results in the overexpression of p16 protein. Whereas in tobacco-

induced HNSCC the abrogation of TP53 and pRb pathways occurs via mutation and epigenetic alterations, in HPV-related HNSCC wild-type TP53 and pRb are functionally inactivated by the viral oncogenes. E7 also induces cellular proliferation by disrupting the activity of cyclin-dependent kinase inhibitors p21 and p27 [48]. E5 is another viral protein that modulates the EGFR signaling pathway by delaying the downregulation of EGFR and increasing the level of EGFR [49]. In summary, HPV infection induces failures in cell cycle checkpoints, which causes genetic instability and, over time, progression of pre-malignant lesions to invasive squamous cell carcinoma.

Importantly HPV status in HNSCC, in particular, oropharyngeal squamous cell carcinoma is an independent prognostic factor for overall survival and progression free survival [1]. Studies have also shown that HPV positive patients demonstrate higher response rates to chemoradiation and an improved overall survival [50,51].

3. Tumor DNA for molecular diagnosis

3.1. Tumor

Comprehensive genomic profiling of HNSCC tumors has demonstrated differences in the mutational profiles of HPV + ve and HPV –ve HNSCCs. With HPV –ve patients demonstrating frequent somatic mutations affecting cell cycle (CDKN2A, TP53 and CCND1), cell survival (EGFR, PIK3CA and PTEN), WNT signaling (FAT1, AJUBA and NOTCH 1) and epigenetic regulation (KMT2D and NSD1). Somatic mutations in HPV + ve patients are markedly different with the most commonly mutated TP53 and CDKN2A genes in HPV –ve patients largely unaffected. Rather, gene mutations in the PIK3CA pathway constitute the most commonly altered molecular structures [7,52]. Overall, the PI3K pathway is the most frequently mutated mitogenic pathway in HNSCC [53], and with the advancement of precision medical approaches, despite randomized clinical trials showing no survival benefit for EGFR tyrosine kinase inhibitors in unselected patients with HNSCC, this pathway offers potential for targeted therapies. For example, an exceptional responder has shown response to erlotinib with a near complete histologic response likely related to an activating MAPK1 E322 K mutation [54]. These results highlight the potential outlier molecular genomics of tumors offers an understanding cancer biology and importantly, the potential to affect our clinical management of HNSCC patients.

3.2. Liquid biopsies

Plasma circulating cell-free DNA (cfDNA) has been successfully utilized in multiple fields of medicine since the mid-twentieth century and identified as early as the 1940's [55–57]. Most notably in circulating fetal DNA collected from maternal plasma to screen for germline mutations [58–60]. Circulating tumor DNA (ctDNA) is cell free DNA that is released from tumors into the circulation. ctDNA can be distinguished from cfDNA on the basis of somatic mutations that result in tumor formation [61,62]. Rapid growth and increased cell turnover are hallmarks of many cancers that inevitably involve cells undergoing apoptosis and necrosis culminating in the release of ctDNA [63–65]. Besides ctDNA that enters the circulation, depending on the anatomic location of the tumor, tumor DNA can also be retrieved from saliva, stool, urine, and Pap smear fluids [66–68]. The amount of DNA shed from a tumor is likely determined by the overall volume of tumor burden, vascularity, invasion into mucosal surfaces, and location.

Prior limitations in the applications of ctDNA for HNSCC have been hampered by the low fraction of ctDNA compared to total cfDNA in circulation, representing < 1.0% of total cfDNA. This has previously been beyond traditional sequencing techniques for the detection and quantification of ctDNA [68, 69]. The advent of digital genomic technologies has revitalized investigation of tumor DNA as a cancer biomarker. Digital polymerase chain reactions (dPCR), beads, emulsion,

amplification, and magnetics (BEAMing), pyrophosphorolysis-activated polymerization (PAP), and Safe Sequencing System (Safe-SeqS) have proven to be highly sensitive techniques at detecting ctDNA in patients [70–73]. Regarding, the use of Circulating Tumor Cells (CTCs), detection of whole cells circulating through the blood stream have been studied in several cancers including HNSCC to utilize them as biomarkers. However, these studies have revealed that there are limitations using CTCs due to technical challenges relating to EpCAM expression and low cell yield [74].

Early work of use of tumor DNA to profile tumors and evaluate tumor burden was done primarily in advanced stage HNSCC patients. In a study evaluating multiple tumor types, 10 HNSCC cancer patients were studied. Somatic mutations were identified in biopsy samples first, then tumor specific ctDNA was isolated in the plasma from 70% of patients [66]. While the previously mentioned study primarily evaluated advanced stage disease, a recent study by Wang et al. [75] highlighted the utility of tumor-specific DNA to detect early HNSCC in multiple anatomical subsites. The study evaluated tumor DNA from saliva or plasma from 93 HNSCC patients. Both plasma and saliva were screened in 47 HNSCC patients with tumor DNA found in 96% of patients. Subgroup analysis determined that tumor DNA was identified in 100% (n = 10) of early (stage I and II) disease and 95% (n = 37) of advanced (stage III and IV) disease. When segregated by primary tumor origin, tumor DNA was detected in all patients with lesions of the oral cavity, larynx, and hypopharynx, and in 91% of patients with oropharyngeal tumors. Overall, this suggests that salivary tumor DNA may be more efficacious for diagnosis in oral cavity cancer and plasma ctDNA in tumors of other anatomic sites. This study also suggested the feasibility in using ctDNA for disease surveillance where post-treatment samples were collected from a total of 8 patients and analyzed for tumor DNA. Tumor-specific DNA was found in 3 patients before clinical presentation of recurrence. Post-treatment samples from the remaining 5 patients yielded no tumor DNA, and at a median follow up of 12 months no disease recurrences had occurred [75].

In HPV-positive HNSCC viral oncogenes can be found in the circulation and secretions, similar to EBV DNA detection in nasopharyngeal carcinoma [76,77]. This means that HPV-positive malignancies can be screened for HPV-specific DNA fragments in place of somatic mutation. A recent retrospective study evaluated the feasibility of using quantitative PCR analysis of HPV-16 DNA in plasma and saliva as marker for OPSCC. Utilizing samples from 81 patients with HPV-positive tumors, combined analysis of pretreatment plasma and saliva yielded a sensitivity of 76% and a specificity of 100%. Post-treatment samples were also analyzed for HPV-16 DNA, and a combined analysis was 69.5% sensitive and 90.7% specific in predicting disease recurrence in 3 years. This early feasibility results demonstrate clinical utility of HPV-16 DNA in screening for OPSCC primary disease and as a predictive tool for recurrence [76]. A more recent evaluation with digital droplet PCR in 8 patients showed a 100% detection rate from plasma ctDNA in a feasibility study evaluating HPV associated carcinomas [78]. The sensitivity of digital PCR of 86% in Wang et al.'s study when evaluating plasma HPV DNA in oropharyngeal carcinoma shows the improved sensitivity of digital PCR and the potential of plasma HPV DNA for the detection of HPV associated oropharyngeal carcinoma [75]. With the identification of plasma EBV DNA being useful for screening for nasopharyngeal carcinoma, and importantly diagnosing the disease at an earlier stage, circulating HPV DNA also has the potential to be utilized and should be evaluated as a screening tool, given the current HPV associated oropharyngeal carcinoma epidemic [79].

These feasibility studies demonstrate that tumor DNA, collected from either plasma or saliva, can be used as a diagnostic tool in HNSCC. Tumor DNA, as determined by the presence of somatic mutations (*TP53*, *PIK3CA*, *CDKN2A*, *NOTCH1*) and HPV viral genes (*E6*, *E7*) are highly specific for HNSCC when isolated from saliva and plasma samples [75,76]. Tumor DNA can also be used to identify both early and advanced HNSCC across multiple anatomic subsites [76]. Interestingly,

tumor DNA appears to be more detectable in early HNSCC than other early malignancies. It is unclear if this reflects differing techniques between studies, or perhaps due to anatomical considerations of the head and neck, such as the increased vascularity and increased accessibility of the region.

Beyond acting as a diagnostic tool, a liquid biopsy utilizing tumor DNA found in blood and other bodily fluids could address many limitations of conventional biopsies, while also opening the door to new paradigms in cancer care. The advantages and applications of a liquid biopsy fall under two broad categories: genetic characterization and tumor quantification.

Much like conventional tissue biopsies, plasma ctDNA - liquid biopsies, can be used as a tool to probe the genetic profile of a lesion. Tumor DNA obtained from bodily fluids will contain exact copies of the mutations present in the tumor, and thus can be used to guide targeted therapies. Because tumor DNA originates from both the primary tumor and metastatic lesions, tumor heterogeneity could be assessed and utilized to design therapeutic strategies targeting both primary mutations as well as minor subpopulation mutations that could confer treatment resistance. This insight into mutational heterogeneity within a tumor could also provide valuable prognostic information and allow for intensification or deintensification of therapy when indicated. The ctDNA can also provide information on the tissue of origin linking genomic information with anatomy with important implications for detection and surveillance of HNSCC [80].

Paramount to the utility of this technique is the sensitivity of current sequencing techniques to detect tumor DNA in liquid biopsies. Because tumor stage correlates with overall tumor burden, staging correlates directly with sensitivity [66]. Amongst multiple different tumor types, tumor DNA sensitivity approaches 100% for advanced stage disease [70]. Contrarily, early stage disease and micrometastatic disease have lower levels of ctDNA and are therefore more likely to yield false negative results related to the lower level tumor burden [81]. Theoretically, the sensitivity of these techniques is limited by the error rate of DNA polymerase, which is generally assumed to be 0.01% [81]. This translates to a detection threshold of 0.01%, and any result reporting a smaller fraction of mutant DNA can be considered negative. Next generation sequencing approaches, such as digital droplet PCR, are under investigation and have shown promising detection thresholds as low as 0.005% [82]. These detection limits are important to keep in mind, because false negative results could limit our detection of early disease and disease surveillance prior to clinically apparent recurrences.

Tumor DNA can also be used in post-treatment monitoring. In current practice, there is no effective way to monitor patients and identify those that have residual disease following surgery or curative therapy. Instead, the risk of residual disease, and therefore the likely benefit of adjuvant chemotherapy, is evaluated with TNM staging. Genetic characterization is emerging as a tool to further risk stratify patients, but it falls short of detecting post-treatment disease [83].

4. Molecular margins

In HNSCC, improved surgical margins analysis is a challenging area that needs to be developed to improve the care of HNSCC. Evaluating margin status following the resection of HNSCC is important, as the presence of positive surgical margins is known to significantly affect overall survival and loco-regional disease-free survival in HNSCC [84]. Even when surgical margins are negative under histological examination with a high accuracy ratio, recurrence rates are still high in HNSCC at about 30% [85–88]. This may be explained by residual cancer cells being left behind, possibly a single cell or a cluster of cells that extend beyond the invasive boundary of the carcinoma, a phenomenon noted as “tumour budding” [89,90] these particular cells are difficult to detect under the microscope during frozen section intraoperatively and may form a nidus for local disease recurrence. Alternatively, fitting the

concept of field cancerization where molecular changes predate phenotypical changes, genetically altered cells that are only detectable by genetic methods may remain and give rise to locally recurrent carcinoma [91]. Molecular assessment of margins of p53 mutations and more recently methylation with quantitative methylation specific PCR (QMSP) and digital droplet PCR have been shown to be of value in assessing local and regional control, and may be useful in prognostication [91–95].

When assessing surgical margins following tumor resection intraoperatively, the location that tumor margins are assessed from are also important. For most assessments two possibilities exist, with either the assessment of the deep tumor bed tissue and mucosal margins, or the mucosal margins and deep tissue of the tumor itself. In both assessments, small representative tissue pieces are sent for frozen section. Recently evidence points to deep margins being more predictive of local recurrence [92,96]. The challenging aspect of assessing the deep margins is that these areas are typically extensive and routine frozen section only assesses an area that the surgeon subjectively considers to be close or positive, subjecting margin assessment to sampling error [92]. To circumvent this problem recently the use of tissue imprinting with nitrocellulose of the entire deep surface has been described for molecular analysis of the deep tumor margin [92].

Another novel way to reliably identify tumor margins using intraoperative real-time imaging using systemic administration of fluorescently labeled antibodies targeting cancer-specific molecules. In HNSCC, a promising intraoperative imaging system under clinical investigation is one using panitumumab, a monoclonal anti-EGFR antibody, conjugated with indocyanine green dye. In a recent in vivo study the tumor was clearly delineated from normal tissue on fluorescence guidance as confirmed by histology. The technique was also able to detect subclinical microscopic residual disease as well as lymph node metastases measuring < 1.0 mm [97]. Fluorescent bioconjugated anti-EGFR nanoparticles or peptides are also being investigated in HNSCC and various other solid tumors such as esophageal cancer, glioblastoma, and epidermoid tumors [98–101]. These nanoparticles or peptides may prove to be more efficient when compared to fluorescent anti-EGFR antibodies due to a shorter half-life and superior tissue penetration and distribution. Further studies are required to elucidate the potential value of these innovative optical molecular imaging techniques in improving surgical outcome and ultimately patient survival

5. Molecular imaging

Radiological imaging has recently joined the array of molecular techniques being employed to diagnose head and neck cancer. Radiologists now offer molecular-based physiologic and functional imaging that boasts an ability to improve the diagnosis and staging of head and neck cancer. Of these techniques, combined positron emission tomography-computed tomography (PET-CT) imaging or conventional PET has been the most utilized in the clinic. PET works by detecting gamma radiation emitted from a tracer that is designed to be a biological component that will undergo specific interactions. By far the most commonly used tracer is fluorodeoxyglucose (18FDG) which is a molecular marker, an analog of part of the glucose pathway, and this produces signal most strongly in cells metabolizing large amounts of glucose. [79] This tracer is also the most commonly used imaging tracer in the diagnosis and staging of HNSCC; and, while sensitivity is often excellent, this technology has many false positives caused often times by benign inflammation. Yet in the clinical setting, PET scans are now routinely used to define the extent of primary disease, the presence of lymph nodal spread, and distant metastasis. PET is also routinely used for follow-up, to assess response to chemotherapy or radiation therapy, and to detect disease spread.

In addition to this typical use, advanced tracers for PET that can molecularly target specific epitopes are available. Researchers are working on utilizing PET tracers that bind to the VEGF receptor and

EGFR receptor that has been shown to be upregulated in HNSCC [102,103]. Researchers have also used DNA precursors, which are incorporated into DNA during repair or division, which identifies areas of cell proliferation. Imidazole agents can identify areas under hypoxic conditions. PET scans are already quite sensitive, but improvements in molecular targeting of tracers will invariably lead to improved diagnostic capabilities that will be used by physicians in the clinic

6. Conclusions

Molecular diagnosis of head and neck cancer is advancing rapidly, but to date few advances have made it into the clinic. Currently tumor DNA from tissue and bodily fluids offers the most advanced and exciting prospect in detecting molecular changes in head and neck cancer. These techniques can be applied to the screening of high risk patients for disease, improvements in staging and prediction of outcome, therapy selection, adequate margin assessment, and disease surveillance. However, to reach significance, these targets require the design of prospective clinical studies to validate and prove efficacy. There is considerable reason for optimism that, in the near future, novel molecular markers will exist that will assist in the diagnosis of patients non-invasively and predict clinical course and response to therapy.

Conflicts of interest

None

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