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Perspectives

The development of a liquid biopsy for head and neck cancers

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ABSTRACT

Developing non-invasive diagnostic tools in the field of head and neck oncology has been a challenge. Analysis of circulating tumour derivatives in a patient's blood has been explored in other solid cancers. This includes analysis of circulating tumour DNA, intact circulating tumour cells (CTCs) and exosomes. These circulating tumour derivatives provide avenues of investigation which can be representative of a patient's primary tumour signature and can be assessed from a patient's blood sample. In advanced stage cancer patients, these tumour derivatives are found in higher amounts, attributed to higher cellular turnover (apoptosis, autophagy), lysed CTCs and sloughing from necrotic tumours. Head and neck squamous cell carcinoma (HNSCC) patients often present with advanced disease associated with a poor 5-year survival of <50%. Outside of sophisticated imaging and clinical examination, there is a lack of available biomarkers to measure disease burden, and/or response to therapy. Implementation of a liquid biopsy in HNSCC through serial blood samples has the potential to detect metastatic events earlier, thereby allowing better selection of appropriate treatment choices, predict prognosis in patients with potentially curable disease, monitor systemic therapies and residual disease post-treatment.

Introduction

Head and neck squamous cell carcinomas (HNSCCs) account for the 7th most common cancer globally and arise from multiple anatomical sites [1,2]. Known risk factors for HNSCCs include tobacco exposure, alcohol consumption, betel nut chewing and infection with oncogenic viruses such as Epstein-Barr virus (EBV) and Human papillomavirus (HPV, in particular HPV 16-18) [1,3,4]. Global incidence of HNSCC is 500,000 new cases annually, with 350,000 associated deaths [1,2,5,6]. HNSCCs can result in high morbidity for patients and can result in difficulty with eating, swallowing and speech. Head and neck tumours may interfere with breathing when obstructing the airway [1,7]. Treatment is also associated with high morbidity for patients and may diminish their long-term quality of life [8,9].

In the past decade there has been a decline in oral, laryngeal and hypopharyngeal cancers with a decreasing trend in cigarette smoking [10,11]. There has however been a marked increase in oropharyngeal squamous cell carcinomas (OPSCC) associated with HPV-16 and -18 infections [11]; biological and clinical features differ between HPV-negative and HPV-positive tumours i.e. HPV-positive OPSCC patients have a better prognosis than HPV negative patients. The high mortality rate in HNSCCs is attributed to late diagnosis, with over 40% of patients diagnosed with Stage IV disease upon initial presentation to clinic; a further 10% of patients present with distant metastasis [1,2,12]. The advanced state of disease and presence of metastasis at diagnosis coincides with an overall 5-year survival rate of less than 50% [2,5].

For patients with early stage HNSCC (stage I and II), the aim is for treatment using a single modality – either surgery or radiotherapy. In more advanced stage disease (III and IV), combined modality treatment is recommended. This may be a combination of surgery and radiation treatment, radiation treatment combined with chemotherapy or all three [9,13,14]. For disease that is not amenable to cure, palliative chemotherapy or best supportive palliative care is offered [1,13,15]. Advanced stage cancer requires more aggressive treatment, resulting in increased morbidity and reducing quality of life. Upscaling treatment provides no guarantee of cure [16]. Earlier diagnosis in these patients is highly desirable in order to reduce morbidity and increase the likelihood of cure.

HNSCC diagnosis utilises the latest imaging platforms (MRI, CT, PET) and histological analysis of the primary tumour biopsy [17], in conjunction with endoscopy and ultrasound guided fine needle aspirates (FNA) used in difficult to access areas. Once

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histopathologic information is available and tumour extent determined, surgery, radiation and/or chemotherapy treatment strategies are devised by a multidisciplinary team [18].

The downside to single-site sampling as with a tumour biopsy for histopathological diagnosis is that of tumour heterogeneity. Evaluating and understanding the importance of heterogeneity within these tumours, may allow for adaptation of usual treatment sequence or refining of treatment protocols. Multiregional and repeated tumour biopsies in order to assess tumour heterogeneity is not practical due to the associated risks of complications, cost, spatial heterogeneity within the tumour and sampling bias [8,14]. Furthermore, studies have shown that single site biopsies are akin to looking through a keyhole of a much larger landscape and that when multiple biopsies were analysed of the same tumour tissue, there was vast diversity [19–21]. This could explain why some cancers are resistant to treatment, or have more regional or systemic metastatic potential. Targetable mutations may be found in only certain areas of the tumour [21], therefore, it is desirable to find a more representative population of the tumour.

Tumour heterogeneity concerns could be addressed by investigating tumour derivatives in the blood, by means of a liquid biopsy. There is a desperate need in the HNSCC field for diagnostic tools which could be used to (i) identify patients at risk of developing metastasis early in order to escalate systemic treatment (ii) assess the tumour heterogeneity so as to tailor treatment options and, (iii) non-invasively track patient treatment and outcomes to monitor for residual disease. This may be possible through investigating circulating cell-free DNA (cfDNA), and in particular circulating tumour DNA (ctDNA) in a patient's blood sample. We believe that blood is an attractive medium for two reasons (i) reduced clonal heterogeneity as cfDNA and CTCs represent a large portion of tumour derivatives and (ii) can be non-invasively sampled compared to a tissue biopsy. The liquid biopsy presents an option where HNSCC patients can be tracked in a non-invasive manner, allowing for serial sampling, informing of the tumour heterogeneity, response to treatment and residual disease [22] (see Fig. 1).

The liquid biopsy

Metastases emerge from the somatic evolution of genetically diversified cancer-cell populations under the selective pressures of an environment [23,24]. The differentiation between normal somatic cells and neoplastic cells is a progressive conversion towards a phenotype which favours tumour growth, invasion and metastasis [23–26]. Intra-tumoral heterogeneity from a genetically diverse cancer cell population, allows for tumour cells with multiple different molecular characteristics [23,24]; conferring different rates of proliferation [24,27,28] invasiveness, [29,30] radiation and drug sensitivities [27,31]. It is these sub populations and residual populations which are refractory to treatment, which may lead to poor patient outcomes and patient deaths [27,32].

The shift towards 'Personalised Medicine' in oncology has facilitated more tailored treatment strategies, with patients stratified on the basis of individual genetic profiles and mutationally activated alleles. However, following implementation of targeted molecular therapies, patients rapidly acquire resistance to treatment and subsequently relapse, despite an initial treatment response. This is evident in recently developed tyrosine kinase inhibitors (TKIs) which target oncogenic versions of *EGFR*, *HER2*, *BCR-ABL*, *BRAF*, *KRAS*, *ALK*, and *JAK2*; with patients developing drug-resistant tumours within 1–2 years of treatment as a result of secondary mutations. In this case, the duration of clinical benefit is invariably short-lived due to rapid acquisition of drug resistance. The mechanism by which drug resistance occurs is still unknown; it is postulated that either secondary mutations develop *de novo* during therapy by mutagenesis or, they are present as minor subclones prior to treatment and have proliferated post-treatment. Furthermore, patients with metastatic disease often have multiple involved sites, but usually only one tumour biopsied and interrogated. While testing might provide information about the genomic landscape of this particular site, it does not reflect the full genomic make-up of the cancer [19,20].

The idea of a 'Liquid Biopsy', analysis of CTCs, cfDNA, and exosomes may provide a means for non-invasive, real-time monitoring of HNSCCs [19,33]. Potential applications of the liquid biopsy have been suggested, namely, prognostic applications in monitoring of response to therapy, emergence of treatment resistance, observation of residual disease and analysis of tumour dynamics and burden in metastatic patients [33–35].

CTCs are rare, metastatic precursors found in the bloodstream of patients with metastatic cancer, existing as single cells, or less commonly as clusters [35–37]. CTCs are shed into the vasculature from primary tumours or distant metastatic lesions, and are thought to contain subpopulations of 'tumour-initiating cells' which are responsible for seeding metastases [28,37–39]. While not the focus of this review, CTCs have been shown to have clinical associations with overall survival and response to therapy [40–42]. Current literature explores the utility of enumeration of CTCs for measuring overall survival and progression free survival, as well as CTC expansion *ex vivo* for drug sensitivity testing, making CTCs an appealing candidate for the liquid biopsy, particularly for detecting HNSCC events [37,43].

Circulating tumour DNA and early detection of head and neck cancer

The cell-free fraction of blood in cancer patients consists of a small variable population of ctDNA, within a larger population of cfDNA [44–47]. ctDNA is thought to be derived from necrotic tumour masses, sloughed tumour cells, lysed CTCs in circulation and inflammatory tissues [35,36]. Much like CTCs, current schools of thought suggest that ctDNA carries somatic mutation fragments which have arisen in primary and/or secondary tumours [35]. Hence, the current utility of CTCs and ctDNA is based on the assumption that they share common somatic mutations and genomic rearrangements as primary/secondary tumours [26,35]. The use of CTCs and ctDNA as a 'Liquid Biopsy' implies that we can obtain a true signature of local and systemic disease with regard to the mutational landscape. Furthermore, the proportion of cancer-derivatives in circulation is likely to better represent tumour dynamics and reduce bias from single-site biopsies [35,48,49].

The idea of using genetic alterations present in tumours as biomarkers for cancer was proposed more than two decades ago [50]. The advantage of using genetic alterations over conventional markers is that genetic changes afford the same specificity of characterised molecular alterations as patient tumour tissue (i.e. SNPs, copy number variation, structural variation). The proposed clinical utility of ctDNA falls within two broad categories; non-invasive characterisation of tumour molecular features (tumour profiling), and quantitation, where ctDNA fractions serve as a surrogate marker for tumour burden [19,33]. Concurrently, ctDNA has been reported to be a highly sensitive genetic biomarker of disease, directly reflecting tumour burden and genetic dynamics in pancreatic [48], melanoma [51], lung [48,49], colorectal [52], breast [53] and prostate cancers [48]. Furthermore, the presence of ctDNA in patients after curative resection or chemotherapies indicates the presence of residual disease and is likely to be a prognostic marker of relapse [33,47,49]. The implementation of a liquid biopsy throughout the course of treatment can help to determine the