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headandneck 5000

Identifying epigenetic biomarkers in cancers of the head and neck

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Scientific outline

Introduction: Head and neck cancer accounts for around 7,000 cases per year in England and Wales (1) and its incidence appears to be increasing (2). Several lifestyle (3) and dietary (4) factors as well as viral infections (5) have been implicated in altering both head and neck cancer incidence and progression. These exposures may also influence DNA methylation patterns which therefore have the potential to provide insight into a) novel exposure or prognostic indicators or b) mechanistic pathways leading to disease.

Epigenetic signatures are marks on the genome which do not change the underlying DNA sequence, integrate genetic and physiological responses to diet and lifestyle, act as early markers of lifestyle-related perturbations, may provide more objective measures of exposure than questionnaires, underpin mechanisms, and may be reversible (6). In addition, epigenetic changes are a hallmark process of cancer (7) and therefore DNA methylation signatures may be a valuable tool for the development of biomarkers for cancer detection and prognosis and as therapeutic targets.

At present, the only prognostic biomarkers currently in routine clinical use for head and neck squamous cell carcinoma relate to HPV16 positivity. Thus, there is a need to identify novel molecular markers that could aid outcome prediction. Furthermore, a better understanding of the molecular mechanisms underlying the differential clinical outcome of HPV-positive and negative cancers could lead to the identification of novel targeted therapies, especially in oropharynx squamous cell carcinoma for which HPV is a strong risk factor (8).

The possibility for identifying novel, potentially modifiable epigenetic biomarkers is growing with developing technologies and epigenetic signatures can be rapidly and reliably measured by high-throughput approaches. Several whole-genome methylation assays have been performed to define the DNA methylation signature of tumour samples (9, 10). In addition, the ability to study cancers

through non-invasive sampling of body fluids is a rapidly advancing development in cancer diagnostics. Methylation markers that are known to persist over time and between tissues may potentially be used to direct treatment (11). In particular, biomarkers identified in saliva hold promise as inexpensive and non-invasive diagnostic and prognostic tools particularly for head and neck malignancies.(12-14)

However, while non-invasive epigenetic profiling is potentially informative with regard to exposure-methylation relationships, it may only reveal limited information with respect to underlying mechanisms of tumorigenesis. As cancer progresses, methylation patterns can change to promote the tumour phenotype and methylation of specific genes could differ significantly depending on the timing and site of tumour sampling. In addition, epigenetic signatures are prone to confounding and, being affected by cancer processes, reverse causation. Hence, it is important to discriminate between epigenetic changes which form part of the causal pathway with cancer from non-causal biomarkers. Given the plasticity of epigenetic biomarkers in response to modifiable exposures any that are causally linked to cancer are appealing targets for preventative interventions. However, irrespective of their causal role, methylation changes may also be informative biomarkers of disease prediction and prognosis, which is clinically valuable for treatment targeting.

Sample and data requirements: We wish to perform epigenetic profiling in those individuals who were diagnosed with oropharyngeal cancer and who have OncoChip genetic data, baseline questionnaire and data capture, as well as blood and saliva samples. Data on HPV status from p16 and tissue block availability will also be incorporated into our sample selection criteria. We therefore request full access to the Head and Neck 5000 database to select and verify sample choice.

Methods**:** Genome-wide DNA methylation will be assessed using the Illumina HumanMethylationEPIC array, run in the MRC IEU laboratories at the Univeristy of Bristol, where an existing project (Accessible Resource for Integrated Epigenomics Studies, ARIES) has established the hardware and informatics for this array. Targeted replication of DNA methylation will be undertaken by pyrosequencing. A Qiagen PyroMark MD and all necessary laboratory equipment and facilities are available in the MRC IEU. Methylation analysis is susceptible to batch effect so we will use batch controls in all assays. Correction for cellular heterogeneity will be applied using the reference free correction method.(15)

We will conduct epigenome-wide association studies for cancer stage and prognosis in oropharyngeal cancers in both non-invasive tissues and cancer tissue blocks where available. Furthermore, we will seek to identify cross-tissue and tissue-specific methylation marks related to prognosis. We will then quantitatively assess, using regression models and ROC curve analyses, the ability of identified methylation biomarkers to predict progression of head and neck cancers.

We will also seek to identify epigenetic signatures related to risk factors implicated in altering both head and neck cancer incidence and progression and we will evaluate the utility of methylation signatures as biomarkers of exposure by generating “scores” that index specific exposures. Related to this is the idea that genome-wide methylation may represent an “exposome” which compiles all exposures that are relevant to define risk profiles and may account for exposure history at critical life stages (16).

In order to link epigenetic biomarkers with both exposure history and cancer stage and progression, we will conduct “meet-in-the-middle” style approaches to aid the identification of these intermediate biomarkers (17). We will also apply innovative causal analysis methods to confidently discriminate between methylation marks that are causally implicated with cancer, and hence are

possible targets for intervention, from non-causal biomarkers which may nonetheless have predictive utility. Given the availability of OncoChip genotype data, analysis will include the application of Mendelian randomization (MR), a now widely applied approach which uses genetic variation to infer the causal nature of associations of modifiable exposures and biomarkers (18-20) with both disease incidence and progression.

We will also assess the clinical implications of our findings in terms in terms of whether causal effects identified are clinically meaningful and whether the biomarkers identified offer utility as exposure and prognosis predictors.

Summary: This project sits within the University of Bristol's Integrative Cancer Epidemiology Programme (ICEP) funded by CRUK which aims to reduce the burden of cancer through the identification of causal risk factors, mechanistic targets and predictive biomarkers. Epigenetic variation has been linked to Head and Neck cancer risk factors and prognosis and may therefore serve both a predictive biomarker and a potential target for intervention. The Head and Neck 5000 cohort provides an extensive collection of biological samples taken from multiple tissues which may be used for epigenetic (DNA methylation) profiling. We aim to identify novel biomarkers for oropharyngeal cancer risk and prognosis by conducting epigenome-wide association studies in both non-invasive tissues and cancer tissue blocks where available. In order to link methylation marks with both exposure history and cancer stage and progression, we will conduct analyses to aid the identification of these intermediate biomarkers and will apply innovative causal analysis methods to confidently discriminate between marks that are causally implicated with cancer from non-causal biomarkers. This will: further our understanding of risk factors and cancer stage and prognosis at a molecular level; improve the characterisation of the dynamic molecular environment involved in tumorigenesis; and may inform treatment targeting, aid outcome prediction and ultimately lead to the development of new treatments.