Exploring **epigenetics**

Dr Matt Lechner leads the Head and Neck Cancer Genome and Epigenome Project. In an engaging conversation, he discusses his research into human papillomavirus-associated head and neck tumours

Could you begin by offering an overview of your principal research aims?

The main aim of my research study is to understand the epigenetic and genetic basis of human papillomavirus (HPV)-associated head and neck cancer, a rapidly evolving cancer in the Western world. This is of particular interest, as patients with HPV-positive head and neck cancer have a better prognosis than patients with head and neck tumours caused by smoking and alcohol abuse. The described difference in the survival rates may be explained by alterations, in particular in the epigenome of tumour cells. This will guide the development of novel biomarkers for this deadly disease.

What may be the reason for the rapid increase of HPV-associated head and neck cancer?

It may be that the epidemiology of the cancer is driven by population changes in sexual behaviour. In the UK, for example, a significant increase in the proportion of people reporting oral sex occurred between 1990 and 2000.

Why has there not been an improvement in survival rates of head and neck cancer over the past three decades?

One of the main reasons for the poor survival rates is that this form of cancer, in particular oropharyngeal cancer, often causes no or unspecific symptoms at early disease stages. Hence, patients often present at advanced disease stages at which treatment is limited to palliative measures only in some cases. Biomarkers for early-stage disease do not exist and relatively few novel treatments have become available over the last decades.

How might epigenetic factors influence HPV?

Epigenetic processes may affect both the viral genome itself and the host cell genome. Modification of normal epigenetic processes in the host occurs during viral infection: for example, compared with HPV-negative head and neck squamous cell carcinoma (HNSCC), HPV-positive tumours show less genome-wide hypomethylation. These epigenetic changes may occur through direct interactions between HPV proteins, eg. E2, E7, and the body's own proteins involved in DNA methylation. Viral oncoproteins – in particular, E7 – can bind and regulate the enzymatic activity of an enzyme that methylates DNA (DNMT1).

You have been using a new approach made possible by recent advances in DNA profiling. What is this new technique?

I used a method that was developed in the Beck lab by Dr Christina Thirlwell at the University College London (UCL) Cancer Institute. By applying a ligation step, it made it possible to use formalin-fixed paraffin-embedded tumour material for large-scale DNA methylation array analysis. For the initial experiment, Illumina 27k arrays were used. I was then able to apply this method to analyse my samples with the new Illumina 450k methylation arrays, and validated this method by an immunoprecipitation technique coupled with next-generation sequencing (MeDIP-Seq).

Can you describe the implications of your HPV-positive and HPV-negative DNA methylation analysis results? How will this inform further research?

My work has contributed to the understanding of epigenetic changes at oncogenic loci associated with the progression of HPV-positive and HPV-negative HNSCC. These may explain the better outcome and survival of patients suffering from this distinct subtype. In the longer term, these data can be expected to be used for the identification of potential diagnostic and prognostic markers, as well as putative therapeutic targets.

Do you collaborate with any other institutions, partners or organisations in conducting this research programme? How are they involved?

In this field of research, collaboration is key. I had the fortune to be trained in the Beck and Boshoff labs at the UCL Cancer Institute, and this allowed me to work in a fantastic environment with a team of enthusiastic and excellent scientists. Among many others, I am now mainly working with Dr Tim Fenton, who is a Rosetree Trust Fellow, and Dr Martin Forster, who is a Senior Lecturer at the UCL Cancer Institute. Together we are taking this project further. I am also collaborating with other institutions at UCL and UCL Hospitals as well as the University of Cambridge in order to investigate aspects of oral HPV infection, with the aim to identify a valid and reliable biomarker for oral HPV disease.

Could you give an insight into how you envision this research will progress over the next two to five years? What would you like to achieve?

My work at the moment mainly focuses on the integration of my research data with independent large-scale datasets. Over the next five years, I will focus on the progression of oral HPV infection to oropharyngeal cancer and understanding the limiting steps in this process. This will allow the development of markers for risk stratification and guide an indication for treatment of precancerous lesions.
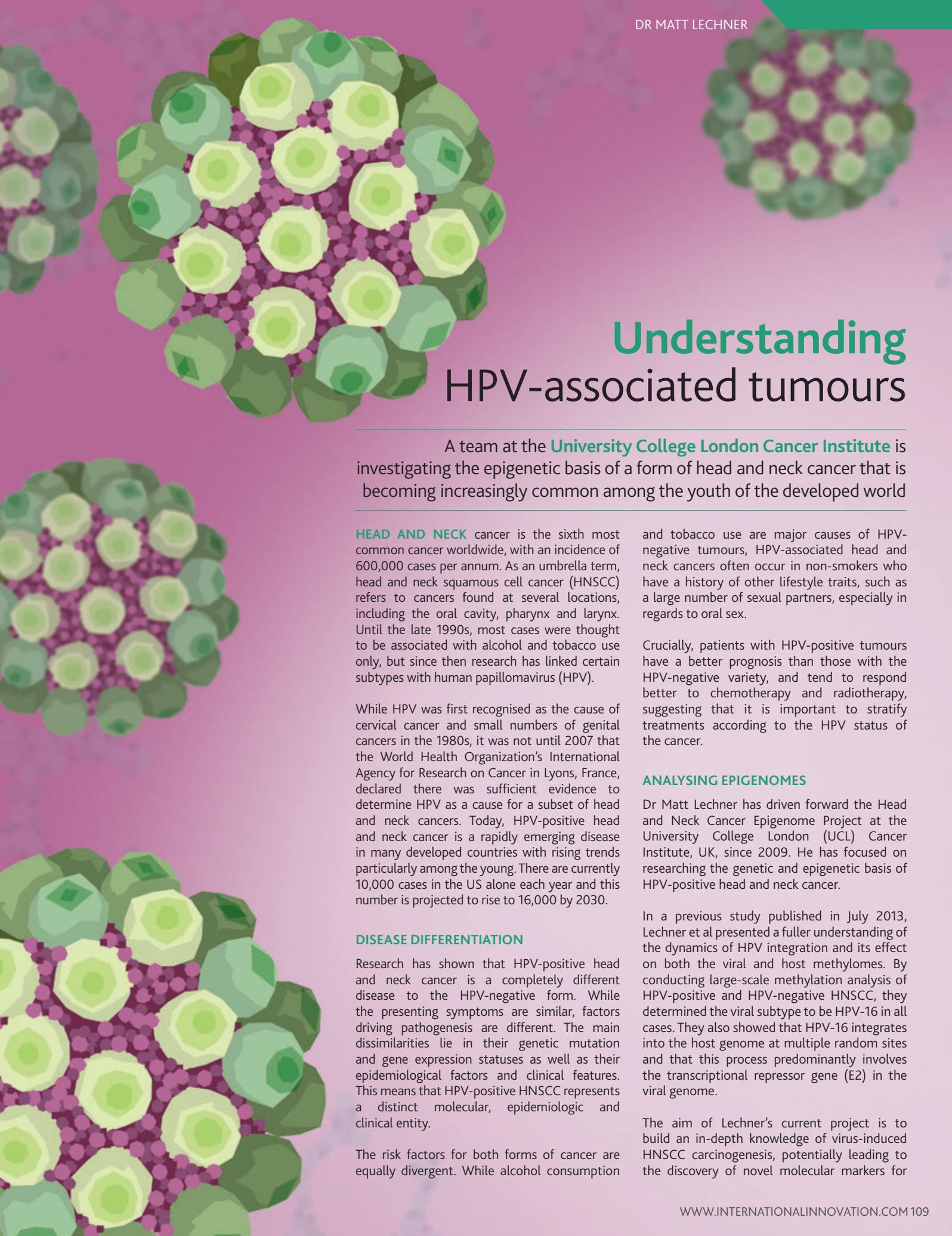
Understanding
HPV-associated tumours

A team at the **University College London Cancer Institute** is investigating the epigenetic basis of a form of head and neck cancer that is becoming increasingly common among the youth of the developed world

HEAD AND NECK cancer is the sixth most common cancer worldwide, with an incidence of 600,000 cases per annum. As an umbrella term, head and neck squamous cell cancer (HNSCC) refers to cancers found at several locations, including the oral cavity, pharynx and larynx. Until the late 1990s, most cases were thought to be associated with alcohol and tobacco use only, but since then research has linked certain subtypes with human papillomavirus (HPV).

While HPV was first recognised as the cause of cervical cancer and small numbers of genital cancers in the 1980s, it was not until 2007 that the World Health Organization's International Agency for Research on Cancer in Lyons, France, declared there was sufficient evidence to determine HPV as a cause for a subset of head and neck cancers. Today, HPV-positive head and neck cancer is a rapidly emerging disease in many developed countries with rising trends particularly among the young. There are currently 10,000 cases in the US alone each year and this number is projected to rise to 16,000 by 2030.

DISEASE DIFFERENTIATION

Research has shown that HPV-positive head and neck cancer is a completely different disease to the HPV-negative form. While the presenting symptoms are similar, factors driving pathogenesis are different. The main dissimilarities lie in their genetic mutation and gene expression statuses as well as their epidemiological factors and clinical features. This means that HPV-positive HNSCC represents a distinct molecular, epidemiologic and clinical entity.

The risk factors for both forms of cancer are equally divergent. While alcohol consumption and tobacco use are major causes of HPV-negative tumours, HPV-associated head and neck cancers often occur in non-smokers who have a history of other lifestyle traits, such as a large number of sexual partners, especially in regards to oral sex.

Crucially, patients with HPV-positive tumours have a better prognosis than those with the HPV-negative variety, and tend to respond better to chemotherapy and radiotherapy, suggesting that it is important to stratify treatments according to the HPV status of the cancer.

ANALYSING EPIGENOMES

Dr Matt Lechner has driven forward the Head and Neck Cancer Epigenome Project at the University College London (UCL) Cancer Institute, UK, since 2009. He has focused on researching the genetic and epigenetic basis of HPV-positive head and neck cancer.

In a previous study published in July 2013, Lechner et al presented a fuller understanding of the dynamics of HPV integration and its effect on both the viral and host methylomes. By conducting large-scale methylation analysis of HPV-positive and HPV-negative HNSCC, they determined the viral subtype to be HPV-16 in all cases. They also showed that HPV-16 integrates into the host genome at multiple random sites and that this process predominantly involves the transcriptional repressor gene (E2) in the viral genome.

The aim of Lechner's current project is to build an in-depth knowledge of virus-induced HNSCC carcinogenesis, potentially leading to the discovery of novel molecular markers for

UNDERSTANDING THE EPIGENETIC LANDSCAPE OF HPV-ASSOCIATED HEAD AND NECK CANCER

OBJECTIVE

To understand the epigenetic and genetic basis/landscape of human papillomavirus (HPV)-associated head and neck cancer, a rapidly evolving cancer in the Western world.

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DR MATTHIAS LECHNER graduated from the University of Innsbruck, Austria, as one of the youngest students nationwide. His MD thesis in the field of mass spectrometry was published and cited in various papers. He completed his family medicine residency training in the UK, Austria, and on a fully funded exchange fellowship programme in Indonesia, where he set up a global health project with the aim to decrease the high mortality from cancer in this region (www.yogyahealth.org). Lechner was awarded a prestigious Wellcome Trust Research Training Fellowship and completed a PhD on HPV-associated head and neck cancer at UCL. Thereafter, he started his surgical training in otolaryngology, currently working at the Royal National Throat, Nose and Ear Hospital, and continued his research work as a postdoctoral research fellow and later as a lecturer at the UCL Cancer Institute. He is a member of the Royal College of Surgeons of England and was awarded a Fellowship from the UK Higher Education Academy (FHEA) in 2013.

risk prediction, early diagnosis and therapeutic targets. The hope is that a deeper understanding of HPV-associated head and neck cancer could allow the therapeutic targeting of affected pathways for a stratified medicine approach.

In order to discern why HPV-positive patients have a better prognosis than their HPV-negative counterparts, Lechner has analysed both epigenomes for differences in DNA methylation and integrated these with messenger RNA expression data. The epigenetic changes at oncogenic loci associated with progression to carcinoma – particularly in the HPV-positive form of the disease – are not well characterised and it is only recent technological advances that have enabled the study of these epigenetic variations at the genome level.

GROUNDBREAKING TECHNOLOGY

As frozen HNSCC samples are hard to source, Lechner was able to validate a method developed in-house that uses paraffin-fixed tumour samples. These samples are readily available and proved to be highly suitable for methylation analysis. In the study, formalin-fixed paraffin-embedded HNSCCs were carefully dissected using a laser system, allowing the enrichment of tumour cells for subsequent analysis. DNA methylation profiles were then generated using two advanced techniques: an array-based method allowing the evaluation of the methylation state of over 480,000 single methylation sites across the genome and a technique in which methylated DNA is immunoprecipitated and then sequenced.

As a result, the team was able to identify differences in the methylation profiles between HPV-positive and HPV-negative tumours. Unsupervised clustering over the differences in methylation profiles showed that the samples segregated according to their HPV status: "This was a clear indicator that both groups harbour a distinct methylation profile," adds Lechner. "Interestingly, it showed that HPV-positive tumours are heterogenous with regard to this profile."

Lechner was able to cross-validate these methylation data into two sets of independent HPV-positive and HPV-negative samples. For the functional analysis, an HPV-negative HNSCC cell line was transduced with lentiviral constructs containing the two HPV oncogenes (E6 and E7) and the effects on methylation were assayed. Combinatorial ectopic expression of these oncogenes in an HPV-negative HNSCC cell line partially phenocopied the hypermethylation signature observed in HPV-positive tumours and established E6 as the main viral effector gene.

BEYOND HEAD AND NECK CANCERS

In order to further test the effect of HPV on DNA methylation, Lechner and colleagues

integrated publicly available methylation data on cervical cancer (an example of HPV-associated cancer) and lung cancer (an example of smoking-induced cancer) with the HPV-positive and HPV-negative HNSCC samples. This was then used for multidimensional scaling of the datasets.

An overlap of cervical cancer samples and HPV-positive HNSCC samples was observed, which is likely to be due to the fact that HPV causes

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the same derangements in both tissues: "This confirmed the observed DNA methylation signature to be HPV-specific and tissue-independent," summarises Lechner.

TREATMENT FOR THE FUTURE

The biomarkers based on Lechner's data, along with the recently obtained genetic, epigenetic and gene expression data on HPV-positive and HPV-negative HNSCC, will undoubtedly serve to advance the field of head and neck cancer diagnosis.

For Lechner, the goal is that an understanding of the methylation differences between tumour types will allow patients to be stratified according to the HPV status of their cancer, ensuring they receive the best possible treatment. "In my ongoing research, I am integrating methylation data with genetic data and expression data, and validating findings in large independent datasets," he explains. "These data will guide the development of diagnostic and prognostic biomarkers for this type of disease."