New perspectives to respiratory tract cancers

MW Marcus¹, Y Chen¹, T Liloglou¹, JK Field¹*

Abstract
Respiratory tract cancers (RTCs), which include the lung and head and neck squamous cell carcinoma, are public health problem with incidence and mortality rates among the highest in the world. In order to reduce the health burden of RTCs, effective public health measures should be put in place. A strategy to achieve this goal could be explored using risk prediction modelling. DNA methylation and microRNAs (miRNAs) have rapidly emerged as potential biomarkers for assessing the risk of early development and therapeutical stratification of various types of cancer. The integration of biomarkers in the development of risk prediction algorithm for estimating the individual probability of developing RTCs may facilitate early detection and improve clinical management of this deadly malignancy. This review presents the current literature on the identification of high-risk individuals, risk prediction models, biomarkers (DNA methylation and miRNAs) and the integration of biomarkers in current and future clinical trials of RTCs.

Introduction
Respiratory tract cancers (RTCs) comprise the lung and head and neck squamous cell carcinoma (HNSCC) and belong to the most common cancers, with an estimated 2.72 million diagnosis (21% of all cancer diagnoses) and 2.12 million cancer deaths (28% of all cancer deaths) per annum.1 Of these cancer types, lung cancer remains the most common malignancy with an estimated 1.61 million new cases (12.7% of all new cancers) and 1.38 million deaths (18.2% of all cancer death) annually.1 Lung cancer is the leading cause of cancer-related deaths worldwide, with mortality rate exceeding that of colon, breast and prostate cancer combined.2 With increase longevity, the incidence of lung cancer is expected to increase in the coming decade. Although smoking is regarded as the major aetiological risk factor for lung cancer, global statistics have estimated that 15% of lung cancer in men and 53% in women are not attributable to smoking.3 Epidemiological studies have identified other risk factors including occupational and environmental conditions such as asbestos, radon or contaminated dusts and particulate matters,4 chronic obstructive pulmonary disease (COPD) including bronchitis and emphysema,5 tuberculosis6, family history of lung cancer7 and other cancers.8 HNSCC includes malignant tumours originating from various sites of the upper aerodigestive tract, the paranasal sinuses and the salivary gland and accounts for about a million new cases and 320,000 deaths annually.9,10 The major risk factors are alcohol and tobacco smoking.10,11 Geographical variations in incidence and sites of tumours for men and women have been reported with alcohol and tobacco showing synergistic interaction.12 Other risk factors implicated in various epidemiological studies include obesity13, betel quid chewing14, mate beverage12, low social economical factor15, gastroesophageal reflux16 and viruses such as human papillomavirus (HPV)17, herpes simplex virus (HSV)18 and Epstein–Barr virus (EBV)19. Although RTCs are malignant tumours arising from a variety of sites, their shared common aetiologies, risk factors and molecular characteristics could be explored to build risk prediction models.

Identification of high-risk individuals
The term risk could be defined as an inherent probability of a healthy individual developing a certain disease in the course of time. Risk factors are characteristics, nature or nurture that increases the probability of the development of a certain disease. Risk prediction for RTCs is the process of identifying the risk factors and combining them into probability estimates of developing RTCs, either over a discrete period of time or over a lifetime.20 Risk prediction has been around for a while and its objective is to target high-risk individuals for screening and prevention. It is cost-effective and can be tailored to optimise the clinical balance of benefits and harms of screening. The identification of high-risk individuals for RTCs will involve identifying the risk factors for the various sites affected by this cancer type. However, the emergence of technologies that measure genetic information and other molecular and physiological attributes of individuals is expected to facilitate the development of new and robust risk prediction models for high-risk individuals.

Risk prediction models
The development of risk models can be categorised into three stages: (1) derivation stage, (2) validation stage and (3) impact analysis stage.17 The derivation stage involves the identification of (potential) risk factors using statistical analysis. Usually, variables that are statistically and clinically associated with the risk of RTCs at a 5% level in univariate analysis are...
The discriminative performance of a robust method for risk prediction.

Biomarkers
Biomarkers, also known as molecular markers or signature molecules, are biological molecules found in blood, other body fluids or tissues and can be used as a prognostic tool for future health status either for individual or cohorts in clinical trials. They are good indicators of normal biological processes, pathogenic processes or pharmacological responses to therapeutic intervention. Biomarkers can also provide us with real-time clinical information that can be used as a surrogate of individual’s current status, which is superior to self-reported responses to questionnaire data or medical records that are subjected to information bias. A major advantage of some biomarkers is that they can be collected during routine clinical practice; collection is non-invasive, and biomarkers can be obtained at low cost.

Epigenetics, defined as heritable changes in gene expression that are not due to any alteration in DNA sequence, is one of the most rapidly expanding fields in biomedical research. Advancement in this field has shed more light on the role of epigenetic programming in normal biological processes as well as in disease pathogenesis. Epigenetic modifications can be grouped into DNA methylation, miRNAs, covalent histone modifications and nucleosome remodelling. In this report, we will focus on the potential of exploiting DNA methylation and miRNAs as biomarkers for RTCs.

Recent advancement in epigenetics and molecular techniques has led to the development of many biomarkers that may facilitate clinical management of this malignancy. For example, Nakahara et al. have reported aberrant p16 methylation in oral squamous cell carcinoma (OSCC) patients using methylation-specific polymerase chain reaction (MSP). In their study, they obtained tumour specimens from 17 patients with OSCC and found aberrant methylation of the p16 gene in 11 out of 17 (64.7%) patients. Shaw et al. were the first to use pyrosequencing techniques for quantitative methylation analysis to investigate the presence of a CpG island methylation phenotype (CIMP) in OSCC. In their study, they obtained tumour tissue, control tissue and peripheral blood lymphocytes from 74 patients with OSCC and investigated allelic imbalance using a multiplexed panel of 11 microsatellite markers; they found histopathological staging and grading to be correlated with genetic and epigenetic aberrations. Viet et al. used methylation array analysis to demonstrate highly methylated gene loci as a potential biomarker in the saliva of OSCC patients before and after oral cancer resection.

Sputum cytological atypia collected during routine clinical practice has been reported to be predictive of future development of lung cancer. Nunes et al. reported the detection of oral oropharyngeal cancer by microsatellite analysis in mouth washes and lesion brushings. Ovchinnikov et al. used MSP to demonstrate hypermethylation of the tumour suppressor gene in the saliva of HNSCC patients. Likewise, Carvalho et al. also showed that serum and saliva rinses were (potential) biomarkers of HNSCC in a case control study of 211 HNSCC patients and 527 controls. miRNAs are short non-coding post-transcriptional RNA molecules that are involved in the regulation of gene expression. They have been implicated in biological processes such as development, cell proliferation, differentiation and apoptosis. Epigenetic reprogramming of cancer cells has shown aberrations in miRNA profiles and thus their potentials as biomarkers for diagnostic, prognostic and therapeutic purposes. Studies have
reported the potentials of using miRNA in circulating body fluids such as plasma, serum or sputum as potential biomarkers for early detection of cancers. Tran et al. explored the role of miRNAs in a cell line of HNSCC using an oligonucleotide array platform. Of the 261 miRNAs studied, 33 miRNAs in the array were found to be highly expressed and 22 showed low levels of expression in all cell lines. Two miRNAs, miR-21 and miR-205, were observed to be highly expressed in all cell lines. Chang et al. carried out an oligonucleotide array study of expression in all cell lines. Two miRNAs, miR-21 and miR-205, were found to be highly expressed and 22 showed low levels of expression in all cell lines. Their study demonstrated that the signature of miRNA mir-21 was overexpressed and that the transfaction of mimics of mir-21 enhanced cell growth; inhibiting it resulted in a decrease in cell proliferation. Kimura et al. examined the expression of miRNAs in HNSCC and esophage squamous cell carcinoma (ESCC) compared with that in normal squamous epithelia as well as malignancies of other organs using real-time quantitative RT-PCR in formalin-fixed paraffin-embedded cancer tissues and paired normal epithelia. In this study, Mir-21 was upregulated in HNSCC and ESCC than in paired normal squamous epithelia. In another study, Matsushima et al., in order to reveal miRNAs’ signatures of ESCC, analysed miRNAs extracted from ESCC cell lines using the miRNA microarray and confirmed significant alterations by quantitative RT-PCR using miRNAs extracted from cell lines or patients’ esophageal biopsy tissues. In their study, they found that mir-205 and mir-10a were significantly altered in cellular expression and that they might be specific for ESCC with potential roles in pathogenesis.

Integration of biomarkers in current and future clinical trials of RTCs

In clinical trials, biomarkers can form the basis of surrogate end points, which can substitute for a clinical endpoint based on epidemiological, therapeutic, pathological or other scientific evidence. Furthermore, biomarker measurements can help explain empirical results of clinical trials by relating the effects of interventions on molecular and cellular pathways to clinical responses and therefore facilitating the understanding of disease pathogenesis. The emergence of technologies that measure biomarkers has revitalised the quest for risk predictions. Because malignancy is most commonly diagnosed at a late stage resulting in poor patient survival, the use of biomarkers for early detection of RTCs and clinical intervention could significantly improve the poor survival rate.

Biomarker design and implementation can be categorised into preclinical and clinical stages. The preclinical stage involves discovering, constructing marker panels, retrospective training sets and validation sets, whereas the clinical stage involves large prospective cohorts. Tumour tissues from various RTCs sites are utilised in the discovery phase. After examination by a pathologist, they are isolated from residual normal tissue to yield at least 75% tumour per sample. First, advanced analytical methods such as microarrays or next generation sequencing are used to identify potential biomarkers. Second, bioinformatical analysis is used to interrogate the potential biomarkers for validation. Third, the frequency of positives in primary RTCs is determined and used for the estimation of power calculation. Highly sensitive and specific robust assays that are able to meet clinical standards should then be designed for those targets with high frequency in tumour tissues and low frequency in adjacent normal tissue. These assays can then be used to screen body fluids from a longitudinal retrospective case control study organised in training and validation sets. Data obtained from the training set are used to define the minimal set of markers required to construct an algorithm and to calculate the diagnostic index. The final panel is then tested on the validation set, in which the ultimate sensitivity, specificity and diagnostic efficiency values are determined.

If successful, biomarker panels will be channelled through a prospective validation phase that will include the assessment of the biomarker in a clinical environment to determine its clinical utility.

Conclusion

RTC s are life-threatening diseases with mortality rates among the highest in the world. The high mortality rate is attributed to the late presentation of RTCs. Identification of individuals at a high risk may be paramount in implementing an effective public health measure. In order to target such individuals, risk prediction models that incorporate (epigenetic) biomarkers are warranted. A highly sensitive and specific risk prediction model may serve as an armamentarium to help identify and counsel individuals at high risk of RTCs and raising awareness for an individual’s risk; this might lead to risk minimising behaviours. Furthermore, risk prediction models may also help in identifying populations at a high risk to be included in prevention trials or to target screening and preventative programmes.

Acknowledgement

This work was supported by the Roy Castle Lung Cancer Foundation.

References


37. Matsuhash K, Isomoto H, Kohno S, Nakao K. MicroRNAs and esophageal