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Laboratory-Clinic Interface

Molecular histology of lung cancer: From targets to treatments

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ABSTRACT

Lung cancer is the leading cause of cancer-related death worldwide with a 5-year survival rate of less than 15%, despite significant advances in both diagnostic and therapeutic approaches. Combined genomic and transcriptomic sequencing studies have identified numerous genetic driver mutations that are responsible for the development of lung cancer. In addition, molecular profiling studies identify gene products and their mutations which predict tumour responses to targeted therapies such as protein tyrosine kinase inhibitors and also can offer explanation for drug resistance mechanisms.

The profiling of circulating micro-RNAs has also provided an ability to discriminate patients in terms of prognosis/diagnosis and high-throughput DNA sequencing strategies are beginning to elucidate cell signalling pathway mutations associated with oncogenesis, including potential stem cell associated pathways, offering the promise that future therapies may target this sub-population, preventing disease relapse post treatment and improving patient survival.

This review provides an assessment of molecular profiling within lung cancer concerning molecular mechanisms, treatment options and disease-progression. Current areas of development within lung cancer profiling are discussed (i.e. profiling of circulating tumour cells) and future challenges for lung cancer treatment addressed such as detection of micro-metastases and cancer stem cells.

Introduction

Lung cancer is the most common cause of cancer-related mortality globally, accounting for more than 1.4 million deaths per year. Outcomes remain poor, despite advances in diagnostics and therapeutics, with a five-year survival rate of 16% in the USA and less than 10% in the UK. Lung cancer is a heterogeneous disease that has historically been divided into two main types: small cell-lung cancer (SCLC) and non-small cell-lung cancer (NSCLC) due to differing disease patterns and treatment strategies. The majority of cases are NSCLC (85%) of which ~40% are adenocarcinoma (AC), ~25–30% are squamous cell carcinoma (SCC) and ~10–15% are large cell carcinomas (LCC) [1–3]. Prognosis is determined by stage at presentation with surgical resection of early stage disease (as well as radical radiotherapy) offering patients the higher probability of cure; unfortunately the majority of patients are diagnosed at an advanced stage of their disease, leading to poor outcomes.

Understanding the unique genetic driver mutations of lung cancer as well as the altered expression of biomolecules (proteins/mRNA) that either cause (or are a consequence) of lung cancer is a key goal in cancer research. This will enable development of treatments, patient stratification and monitoring strategies, enabling outcomes such as the detection of disease recurrence following previous treatment (equivalent to minimal residual disease-assay based approaches in leukaemias) [4].

Lung-cancer profiling and high-throughput sequencing-techniques

In the era of personalised medicine there is a pressing need for the elucidation of the mechanisms underlying lung cancer development both to identify new drug targets as well as to provide more sensitive and specific biomarkers to assist early diagnosis and treatment. Analysis of DNA-sequence (genomics), regulation of gene expression and alternative splicing (epigenomics and functional

Abbreviations: LC, lung cancer; AC, adenocarcinoma; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; LCC, large cell carcinoma; SCC, squamous cell carcinoma; EGFR, epidermal growth factor receptor; KRAS, Kirsten Ras; SNP, single nucleotide polymorphism; GWAS, genome wide association study; aCGH, array comparative genomic hybridization; miRNA, micro-RNA; CSC, cancer stem cell; ABCG2, ATP-binding cassette transporter; EML4-ALK, echinoderm-microtubule-associated protein-like-4 anaplastic lymphoma kinase; SPN, solitary pulmonary nodule; EMT, epithelial-to-mesenchymal transition; PIK3CA, catalytic subunit of PI3-Kinase; MET, hepatocyte growth factor receptor; TKI, tyrosine kinase inhibitor; PFS, progression free survival; OS, overall survival; OR, estimated risk odds-ratio.

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genomics), mRNA expression (transcriptomics), protein expression and post-translational modifications (proteomics) and metabolite levels (metabolomics) have had considerable impact upon the study of a wide range of human diseases. A description of the methodological aspects of novel high throughput techniques is beyond the scope of this review, however, the reader is referred to the following reviews for genomics [5,6]; functional genomics and transcriptomics [7,8]; proteomics [9–11] and metabolomics [12–14].

The following sections outline the potential for such studies to impact upon key questions relating to lung cancer oncogenesis, personalised therapy options and the mechanisms underlying drug resistance. These aspects are considered for all the major forms of lung cancer. Finally, future areas of research into lung cancer are considered including the development of non-invasive markers for therapy monitoring, the potential for "liquid biopsy" approaches (circulating tumour cells) and the detection and profiling of micro-metastases and cancer-stem cells.

Heritable polymorphisms within lung cancer: their influence upon genetic instability and clonal-evolution within lung-cancer

Lung cancer is one of the most heavily mutated and genomically altered cancers [15]. Recent high throughput genomic sequencing studies within adenocarcinomas identified as many as 12 mutational events/megabase and 18 genes were identified as significantly mutated with a q-score $\geqslant 1.0$ ($\leqslant 10\%$ false discovery rate) [16]. Adenocarcinomas (AC) and squamous cell carcinomas (SCC) have a protein-altering mutational rate of 3.5 and 3.9 mutations per Mb respectively, figures which contrast with other cancers for which the mutation rate is lower (e.g. prostate cancer for which the mutation rate is 0.33 per Mb) [15,17].

The high-degree of genomic instability within lung cancer has several origins. Exposure to the complex mix of chemical carcinogens within tobacco smoke plays a role as does inactivation of DNA repair pathway genes. There is interplay between the two mechanisms as smoking-induced DNA damage may itself inactivate DNA repair genes. Genome wide association studies (GWAS) within NSCLC have also identified a series of DNA-repair capacity (DRC) genes with potentially inactivating DNA-repair SNPs, associated with increased risk of developing lung cancer. Evidence that these SNPs play a role in altered rates of DNA repair has been obtained for ERCC2/XPD were the presence of polymorphisms correlated with response-rate to radiotherapy-induced DNA damage within NSCLC [18] as well as a correlation of ERCC2/XPD SNPs with repair of sister-chromatid breaks and gaps following X-ray irradiation of women with high-susceptibility to breast cancer [19]. The three most significant genes predicting this DRC phenotype were:

- ERCC2/XPD – an ATP-dependent-5′–3′-helicase component of the basal transcription factor IIH complex responsible for repairing bulky DNA adducts typical of those formed by cigarette smoke for which SNP polymorphisms were detected within 25.5% of all DRC cases [20],
- Phosphatase and actin regulator-2 (PHACTR2)-a regulator of the cytoskeleton, with SNP polymorphisms detectable within approximately 25.6% of all DRC cases [20] and,
- Dual specificity protein phosphatase-1 (DUSP1)-a negative regulator of the MAP-kinase pathway [20] which functions to regulate the cellular apoptosis rate following UV-induced DNA damage [21] with SNP-polymorphisms present within 24.6% of DRC cases [20].

There is evidence for a hereditary component within lung cancer however the contribution to risk is quite small. Studies within lung cancer cases arising within never-smokers have identified several genes within carcinogen-metabolism, DNA-repair and inflammatory pathways which harbour SNPs with estimated risk odds-ratio OR [95% confidence interval] ranging from 0.36 [0.17–0.77] to 5.09 [1.39–18.67] (reviewed in: [22]). Epigenetic promoter methylation has also been studied for identification of somatic mutations with increased prevalence in lung cancer patients compared to controls. Whilst much remains to be discovered in this area a case-controlled study of sputum samples from high-risk individuals (frequent smokers) and a control cohort matched as closely as possible for potential confounding factors [23] identified a 14 gene detection panel with altered methylation including a six gene panel ($p16INK4a$, death-associated protein kinase-DAPK; tumour suppressor RASSF1A, paired-box protein-5-PAX5; O^6-methylguanine-DNA-methyltransferase MGMT and transcription factor GATA5) associated with a >50% increased lung cancer risk [23]. Partial replication of the results from this promoter-methylation study has subsequently been reported within two independent patient groups recruited at different clinical centres, with 5 genes displaying consistent methylation and disease-association across all patient cohorts [24]. To date the most frequently hypermethylated promoter observed within lung cancer sputum samples is the p16 tumour suppressor gene, which is found to be hypermethylated within 25–74% of lung cancer patients [25]. Despite these promising early results there are still significant technical hurdles to be overcome in the adoption of sputum-based methylation analysis [26]. The inconsistency of results from promoter hyper-methylation studies between different groups may reflect the use of methylation-specific PCR as a detection method and the potential for false positive results with a greater number of PCR-cycles [26]. Despite progress there is still an insufficient understanding of lung-cancer susceptibility genes and risk factors such as the role of ethnicity.

Molecular alterations in adenocarcinomas (AC)

Detection of mechanisms driving lung oncogenesis as potential therapeutic targets

Gene fusions and alternative splicing

One of the most studied molecular alterations at the gene level within lung cancer is the existence of cancer-specific gene fusions [27–33], which are rare events within lung cancers, however results from ongoing gene sequencing studies may alter this reported frequency of occurrence.

A high proportion of the fusion proteins within AC encode tyrosine-kinase (TK) domains fused to dimerization domains (leucine-zipper and coiled-coil domains). In a large scale sequencing analysis of over 200 adenocarcinoma samples 8 chimeric tyrosine kinases were identified (including EML4-ALK, KIF5B-RET, CD74-ROS1 and SLC34A2-ROS1 fusions), as well as the novel fusion genes CDC6-ROS1, FGFR2-CIT, AXL-MBIP and SCAF11-PDGFRA-all of which involve fusion of tyrosine-kinase domains to dimerization units [32,33].

EML4-ALK fusions have been detected at low frequency within western populations, however rates of 6.3% of AC cases (and 14.2% of SCC cases) within a recent study of Chinese NSCLC patients have been reported [31]. Patients with EML4-ALK fusions are currently treated with the drug crizotinib (a tyrosine kinase ALK inhibitor approved by the FDA [29]) and this has been demonstrated to be both well tolerated and to provide clinical benefit to patients with the EML4-ALK fusion gene [29,34]. In a phase-III clinical trial of crizotinib vs. chemotherapy, crizotinib demonstrated improved performance status (PFS) compared to chemotherapy (7.7 months vs. 3.0 months) and improved response rates (65% vs. 20%,