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Title: The molecular approach to diagnosis in lung cancer.

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Introduction

Lung cancer is the biggest cause of cancer death in the UK (CRUK, 2014). It is a biologically very diverse disease, and shows striking variation seen in histological appearances, which are reflect high levels of genomic changes with concomitant diversity of tumour cellular biology (reviewed in Shames and Wistuba, 2014). Despite this, until a decade ago, a simple classification into two categories, small cell or non-small cell, was the only one relevant to disease management. Small-cell carcinoma generally gave a good initial response to chemotherapy, whereas only non-small cell disease was amenable to surgical cure and there was no clinical reason for pathologists to attempt further classification of non-small cell carcinomas. This review will examine the subsequent developments in lung cancer diagnosis and look forward to how emerging technologies and improved understanding of tumour biology are likely to further transform the pathological diagnosis of this disease.

Improving the subclassification of lung cancer

The first major recent advance to improve lung cancer diagnosis came with the discovery that new chemotherapy regimens were of greater value in adenocarcinoma compared to squamous cell carcinoma (Scagliotti et al, 2009), the differentiation between the main types of non-small cell carcinoma becoming important. As a result pulmonary pathologists began to seek more specific diagnoses, but this proved difficult given the very small biopsies often available. Although many cases of non-small cell carcinoma lack the morphological features required to allow for accurate tumour typing, particularly on small, crushed biopsy specimens, a panel of 2 or 3 immunohistochemical markers applied to the tumour tissue sample allows identification of a tumour as either adenocarcinoma or squamous cell carcinoma in the majority of cases. In this way, relatively simple tests of gene expression, coupled with traditional histopathology, permit further biological subclassification of tumour types.

A further advancement came with the recent and ongoing characterisation of the range of genomic changes within tumour cells (Cancer Genome Atlas Research Network, 2014). In particular, 'driving mutations', or changes that affect the activity of a single gene that facilitates the malignant phenotype, have been identified. Adenocarcinomas in particular contain recurrent mutations that are amenable to mutation-specific therapies. For example, around 20% of lung adenocarcinomas have been shown to contain driving mutations in the EGFR gene. Gefitinib, is a small molecule that blocks the EGFR-encoded tyrosine kinase receptor, and it shows strong antitumour activity in sensitive cases (Lynch et al, 2004). It was approved for use in the EU in 2009. This produced a new diagnostic challenge, as it became imperative to identify adenocarcinoma cases with EGFR mutations that might benefit. In addition, the KRAS gene must also be examined, as tumours driven by KRAS are insensitive to anti-EGFR therapy, even if they carry EGFR mutations. The other widely adopted test is for translocation of the ALK gene, which confers sensitivity to another tyrosine kinase inhibitor, crizotinib, in around 2% of UK patients.

Through these major advances in lung cancer treatment in recent years, the complexity of making an accurate pathological diagnosis in lung cancer biopsies has significantly increased (figure 1).

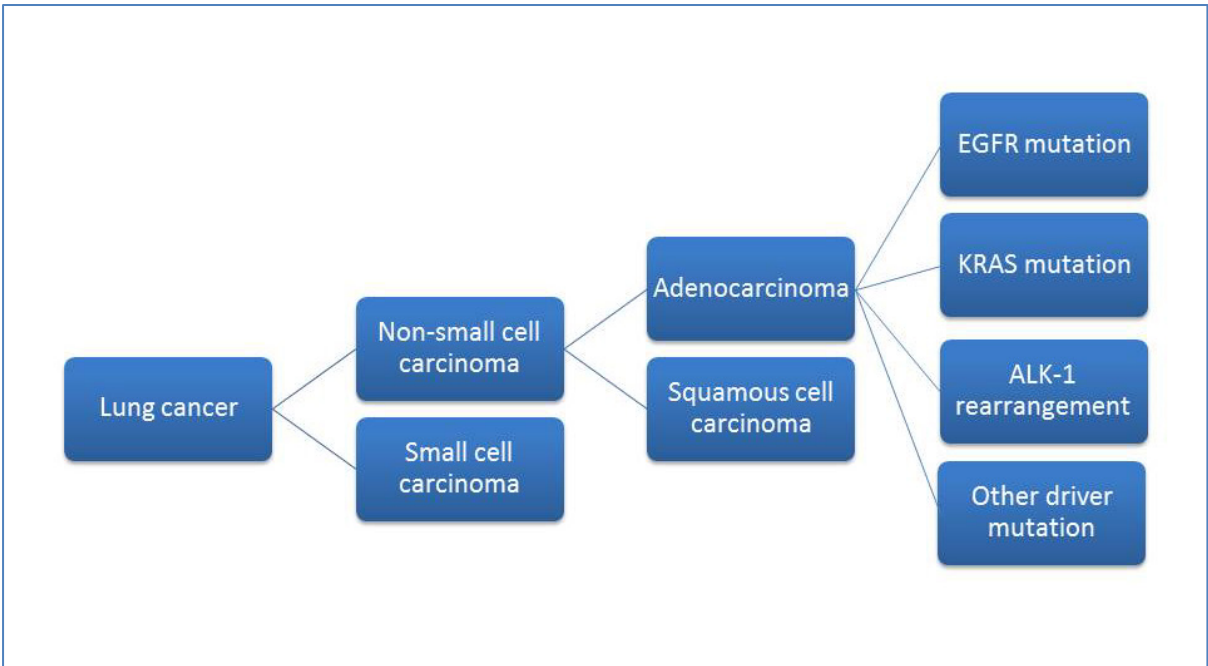


Figure 1. Sub classification of lung cancer including clinical relevant molecular changes.

DNA testing services have now been established in many tertiary referral centres, and they operate with a rapid turnaround to meet the clinical demand. Widespread molecular characterisation of lung cancers for diagnostic purposes has also produced data, which when collated between multiple clinical laboratories, has helped develop a profile of recognised driver mutation frequency in lung adenocarcinoma (figure 2).

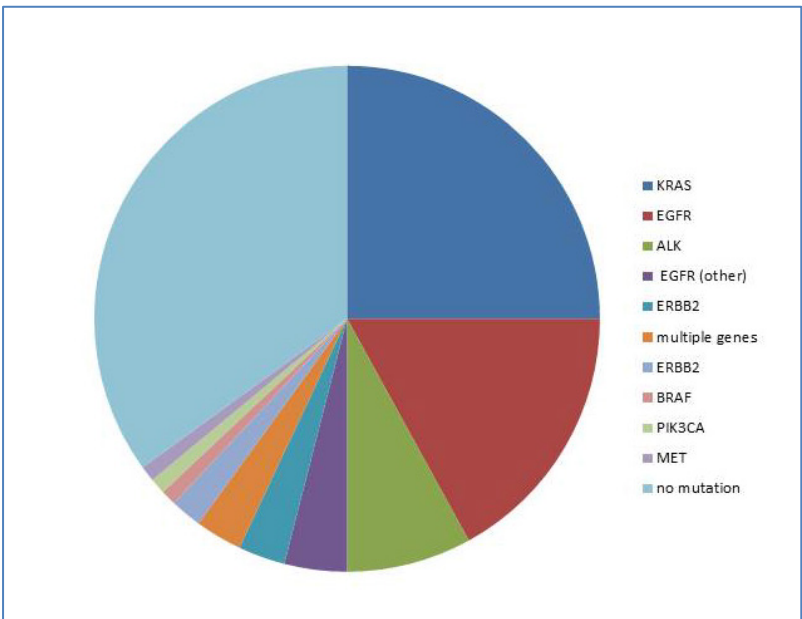


Figure 2. Frequency of identified driver mutations in 733 lung adenocarcinomas (data from Kris et al, 2014).

Cancer genomics in clinical practice

This routine testing for molecular changes in lung adenocarcinomas represents the start of a new era of cancer diagnostics. A great many gene-specific therapies, targeted against a large number of oncogenes are at various stages of pre-clinical testing or in clinical trials. In order to effectively direct these expensive therapies, the range of molecular tests is set to increase dramatically. It seems likely that a decade from now, lung cancer specimens will routinely be tested for a large panel of genomic changes, as will other common malignancies.

The technology required for detection of treatable genomic changes was until recently solely used in the research setting. For detection of classical point mutations that activate oncogenes such as EGFR and KRAS, PCR-based methods predominate. These generally provide a binary output for the presence or absence of a particular sequence change, and therefore are only of value when the mutation being sought has previously been characterised. For activating translocations, such as those seen with ALK, the most widely applied test is Fluorescence In Situ Hybridisation (FISH), although in the case of ALK, immunohistochemistry is also of value (Le Quesne et al, 2014). The PCR-based methods and FISH are both technically demanding, and the best results are obtained from specialist laboratories performing large numbers of tests.

It seems likely that next generation sequencing (NGS) will sooner or later be adopted for clinical testing. This method permits the simultaneous testing of hundreds of genomic loci in a single assay, and is not limited to the detection previously described mutations (reviewed in Wu et al, 2013). It can also be used to detect translocations and chromosomal gains/losses. However, it presents bioinformatic challenges; a single NGS run generates many millions of sequence 'reads', and sifting through these data to identify clinically meaningful alterations is not straightforward. Such technical challenges lead to new challenges of standardisation and quality control of testing, as new external quality assurance systems need to be put into place. The issue of how to report and interpret NGS data in a way that is of immediate value to the clinical team also needs to be overcome.

The complication of tumour heterogeneity

Our increasingly sophisticated understanding of tumour biology has implications for the interpretation of molecular testing. A solid tumour is not genetically uniform, and consists of thousands of divergent clones of tumour cells undergoing a process of Darwinian selection. This is probably the reason why initially successful therapies fail; such treatments apply selective pressure, and a few cells that survive because they carry a particular mutation form the basis of tumour recurrence. We must understand how this process operates, and identify mutations that arise early in the evolution of lung tumours, as they offer the best chance for the developments of targeted therapies with effects upon the entire tumour load. But if tumours are internally diverse, how are we to value genetic tests from small biopsies? This question and many others are being addressed by the CRUK-funded TracerX study of heterogeneity in lung cancer (Jamal-Hanjani et al, 2014 and de Bruin et al 2014).

Diagnostic material

As we seek to draw more and more information from diagnostic material, the quality and volume of tissue submitted for pathological assessment becomes increasingly important. From one small biopsy sample pathology departments now need to provide morphological, immunohistochemical and molecular information, whereas a decade ago one H&E stained section from the same sample may have been enough to secure a diagnosis of 'non-small cell carcinoma'. In particular samples need to contain sufficient viable tumour to allow for the extraction of tumour DNA which can be used for genomic characterisation. Because the processing of material for standard histology requires formalin fixation and paraffin embedding, the DNA yield from these samples is lower and of lower quality than an equivalent fresh frozen sample. Current targeted next generation sequencing technologies require around 10 ng of good quality tumour DNA to be extracted from the sample. For the majority of lung cancer patients who do not undergo a tumour resection, it is imperative that clinicians attempt secure sufficient tissue at biopsy for all these purposes. In addition, pathologists are even under even more pressure to make diagnoses from limited tissue, as little can be spared for deeper sections and/or immunohistochemistry. In time, DNA testing may well make many traditional histopathological techniques unnecessary, but for now, for example, there is no reliable DNA-based discrimination between SCLC, adenocarcinoma and squamous cell cancers.

Liquid biopsy – the future of cancer diagnosis?

A final exciting development in cancer diagnostics is the potential clinical application of assays which can detect circulating tumour DNA. Tumours are known to shed their DNA into the circulation when they die, and of course whole tumour cells enter the circulation during metastasis. Cell-free circulating DNA from plasma and DNA from circulating tumour cells have both been used to identify driving mutations in primary tumours (Luo et al, 2014). If these methods can be reliably applied to blood from cancer patients, it may be possible to molecularly characterise clinically apparent disease without resorting to invasive methods. This 'liquid biopsy' approach, if validated, would drastically alter the role of pathology departments, and could have a huge impact on detection and monitoring of many tumour types. This would be of particular value in tumours in areas difficult to biopsy, such as the lung and ovary.

Conclusion

The routine subtyping of non-small cell carcinoma of the lung, and routine clinical molecular testing for lung adenocarcinoma represents a major development in diagnostic pathology practice. The introduction of NGS technologies and the testing of a wider array of lung cancer associated genes is likely to be the next advancement in this field, whilst current studies in lung cancer tumour heterogeneity and circulating tumour DNA may have a major impact on the future diagnostic pathology of lung cancer.

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Key Points

- Lung cancer diagnosis has evolved over the past decade and subtyping of NSCLC is now crucial for optimal treatment selection.
- Testing for specific molecular alterations, such as EGFR mutation in lung adenocarcinoma, is an early step towards universal personalised cancer therapy.
- The molecular testing of routine clinical specimens is likely to transform routine diagnostic pathology practice as new technologies such as NGS are utilised.
- In order to facilitate molecular testing on tumour biopsy material the quality of the tissue submitted for pathology is of increasing importance.
- Our increasing understanding of tumour heterogeneity may increase the complexity of molecular characterisation in cancer diagnostics.
- The eventual clinical translation of methods to detect circulating tumour cells and cell free DNA could revolutionise cancer diagnosis.