The molecular approach to diagnosis in lung cancer

Lung cancer is the biggest cause of cancer death in the UK (Cancer Research UK, 2014). It is a very diverse disease, showing striking variation in histological appearances, which reflects the diversity of tumour cellular biology and high level of genomic changes (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 required for optimal disease management. Small cell carcinoma generally responds well to chemotherapy initially, 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 article examines the subsequent developments in lung cancer diagnosis and looks 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 sophistication of lung cancer diagnosis came with the discovery that antifolate chemotherapy offered prolonged survival in adenocarcinoma but was less effective than existing regimens in squamous cell carcinoma (Scagliotti et al, 2009), making differentiation between the main types of non-small cell carcinoma crucial. As a result pulmonary pathologists began to seek more specific diagnoses, which often proved difficult given the very small amount of biopsy tissue 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 two or three immunohistochemical markers allows probable definitive 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 advance came with the recent and ongoing characterization of the range of genomic changes within tumour cells (Cancer Genome Atlas Research Network, 2014). In particular, numerous 'driving mutations' have been identified; these are sequence changes that affect the activity of a single gene that facilitates the malignant phenotype. Adenocarcinomas in particular contain recurrent mutations that are amenable to mutation-specific therapies with well-tolerated oral drugs. For example, around 20% of lung adenocarcinomas 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 (Massarelli et al, 2007). 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*).

DNA testing services have now been established in many tertiary referral centres which act as 'hub' diagnostic laboratories, and operate with a rapid turnaround to meet the clinical demand. Widespread molecular characterization of lung cancers for diagnostic purposes has also produced data which, collated between multiple clinical laboratories, have helped develop a profile of recognized driver mutation frequency in large populations of lung adenocarcinoma (*Figure 2*).

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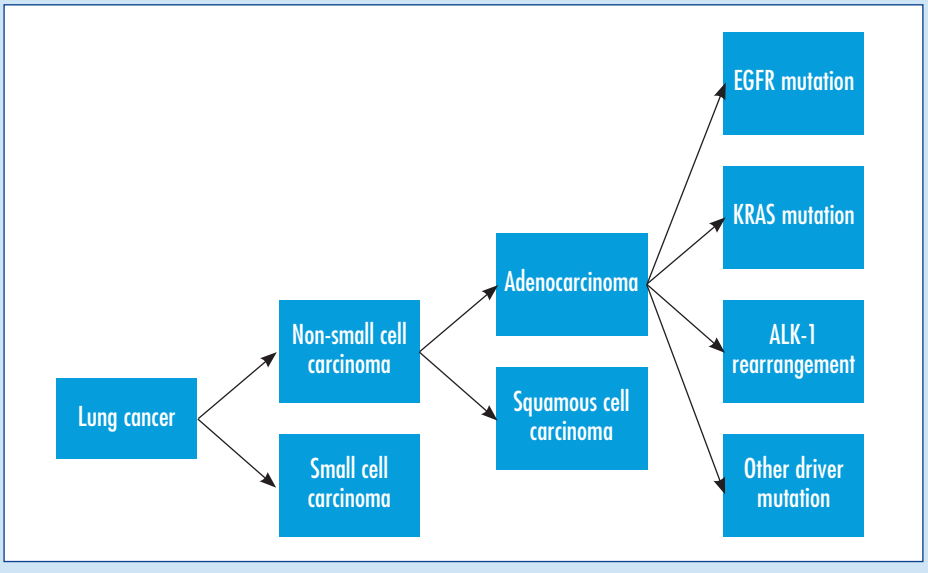
Figure 1. Subclassification of lung cancer including clinically relevant molecular changes.



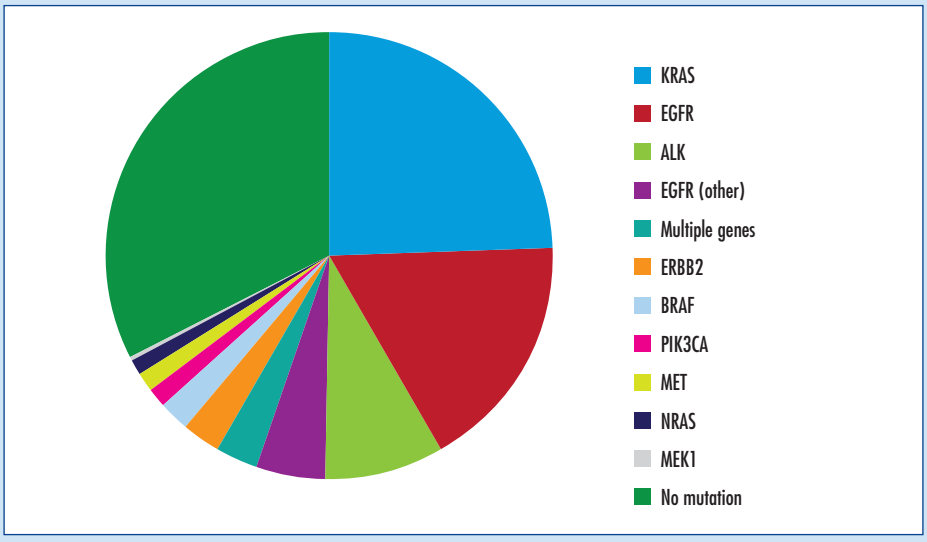


Figure 2. Frequency of identified driver mutations in 733 lung adenocarcinomas. EGFR = drug-sensitizing mutation; EGFR (other) = non-drug-sensitizing mutation. Data from Kris et al (2014).

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 few years from now, lung cancer specimens will routinely be tested for a large panel of genomic changes, as will other common malignancies.

The technologies used for detection of treatable genomic changes were until recently restricted to research. For detection of classical point mutations that activate oncogenes such as EGFR and KRAS, polymerase chain reaction-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 characterized. For activating translocations, such as those seen with ALK, the most widely applied test is fluorescence in situ hybridization, although in the case of ALK, immunohistochemistry is also of value (Le Quesne et al, 2014). The polymerase chain reaction-based methods and fluorescence in situ hybridization are both technically demanding, and the best results are obtained from specialist laboratories performing large numbers of tests.

Next generation sequencing in some form will soon be adopted for clinical testing. This method permits the simultaneous examination of hundreds of genomic loci in a single assay, and as the output is DNA sequence, it is not limited to the detection of previously described mutations (reviewed in Wu et al, 2013). It can also be used to detect chromosomal translocations and gains or losses. However, it presents bioinformatic challenges; a single next generation sequencing run generates many millions of sequence 'reads', and sifting through these data to identify clinically meaningful alterations is not straightforward. Such technical issues lead to new challenges of standardization and quality control of testing, and new external quality assurance systems need to be put into place. The question of how to report data-rich tests such as next generation sequencing in a way that is of immediate value to the clinical team also needs to be addressed.

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; 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 interpret genetic tests from small biopsies? This question and many others is being addressed by the Cancer Research UK-funded TracerX study of heterogeneity in lung cancer (de Bruin et al, 2014; Jamal-Hanjani 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 haematoxylin and eosin 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 cell nuclei to allow for the extraction of DNA which can be used for genomic characterization. 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. Nonetheless, good results can be obtained from a very few cells, and techniques to recover and sequence tumour DNA are improving rapidly.

For now, however, the amounts of tissue available from diagnostic biopsies are often limiting, and pathologists are under more pressure than ever 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 small cell carcinoma, adenocarcinoma and squamous cell cancers, which still depends upon traditional microscopic methods.

Liquid biopsy: the future of cancer diagnosis?

A final exciting development 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 characterize 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 anatomical sites which are difficult to biopsy, such as the lung.

Conclusions

The routine histological subtyping of non-small cell carcinoma of the lung, and routine clinical molecular testing for lung adenocarcinoma represent major developments in diagnostic pathology practice. The introduction of next generation sequencing technologies and the testing of a wider array of lung cancer associated genes is likely to be the next advance in this field, while current studies in lung cancer tumour heterogeneity and circulating tumour DNA may have a major impact on the future diagnostic pathology of lung cancer. **BJHM**

Conflict of interest: none.

KEY POINTS

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

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