The Importance of the Multidisciplinary Team in the Acquisition and Processing of Cancer Biopsy Tissue Samples for Biomarker Testing

BY DAVID SALTMAN, MD, PHD, FRCPC

Introduction

Personalized medicine uses the genetic signature of a cancer cell to diagnose and effectively target activating genes and their corresponding proteins (biomarkers).

The therapeutic targeting of these genetic abnormalities often leads to more effective and safer therapies than seen with nonspecific chemotherapy drugs. For example, the response rates and the improvement in progression-free survival achieved in certain subsets of lung cancer that harbour either an epidermal growth factor receptor (EGFR) mutation or an anaplastic lymphoma kinase (ALK) fusion gene is far greater with EGFR and ALK inhibitors than seen with cytotoxic chemotherapy drugs.[1,2]

Similar results are also seen in a number of other cancer types, which historically have been very difficult to treat when in an advanced stage.

At the cornerstone of personalized medicine is the requirement for the accurate, cost effective and easily reproducible identification of the genetic abnormality that is causing the cancer to grow and will be the target for these novel therapies.

A biomarker for cancer can be defined as genetic material (DNA or RNA) or protein that can be isolated from a tumour and is indicative of a normal or abnormal process. While prognostic markers may indicate the probability of a benefit from a treatment intervention, predictive markers are objective indicators of the sensitivity or resistance of a tumour to a specific therapy that is designed to target that gene or protein.

The detection of biomarkers in cancer biopsy samples may not be possible without an adequate volume of high quality tumour tissue.[3] The pathologist's role in this process is obvious but the involvement of the other members of the multidisciplinary team and effective communication between stakeholders is also critically important. This article will use non-small cell lung cancer as a paradigm for the steps that are required for providing the pathologist with cancer biopsy samples for biomarker testing.

The Role of the Respirologist and Thoracic Surgeon

Patients with lung cancer may first enter the healthcare system either with a visit to their family physician or the emergency department. Symptoms such as cough, bloody sputum, shortness of breath and chest pain usually precipitate a cascade of investigations including chest x-rays and CT scans. Abnormalities seen on imaging studies suggestive of a lung cancer will then result in a referral to a respirologist or thoracic surgeon for biopsy confirmation of the diagnosis.

The detection of biomarkers in cancer biopsy samples may not be possible without an adequate volume of high quality tumour tissue.

Unless the patient has advanced disease evident on physical examination or imaging studies, the first attempt at procuring a biopsy sample is often performed by bronchoscopy. If the tumour can be safely visualized within the airways, then the bronchoscopist will attempt a biopsy using tiny forceps. More than one biopsy fragment may be obtained but the samples are often small. Tumours that are outside the airways can be biopsied by directing the bronchoscopist's needle through the wall of the airway with the use an endobronchial ultrasound or EBUS.[4]

Cancer cells can also often be obtained using exfoliation by brushing or washing the involved airways (bronchial lavage) with a saline or salt solution and collecting the fluid for cytology. The number of cells obtained is often very diluted, requiring the concentration of the material by special techniques to allow the pathologist to have adequate cells to examination microscopically. If the amount of the tumour cells in a bronchoscopic biopsy or obtained by bronchial brushing or lavage is small, then it may impact the pathologist's ability to make a diagnosis of cancer, to sub-classify the tumour or test for biomarkers.

Bronchoscopy has a higher sensitivity for the diagnosis of central lesions and a low sensitivity for the diagnosis of peripheral lesions. Lung cancers that are in the peripheral lung zones that are not accessible by bronchoscopy are usually referred for transthoracic needle biopsy (TNB) under image guidance.

The Role of the Interventional Radiologist

With the exception of tumours that can be easily felt and safely biopsied without imaging guidance or sampled by a surgical procedure with direct visualization, many biopsies, whether intrathoracic or extrathoracic, are done with the assistance of imaging. The two most commonly used imaging techniques for obtaining tissue biopsies include ultrasound and CT scanning.

Image guided sampling of tumours for lung cancer diagnosis and molecular testing are performed using either fine needle aspiration (FNA), or by utilizing a core needle biopsy (CNB). FNA is rapid, cost effective and safe.[5,6] The procedure involves inserting a small diameter needle into the tumour and aspirating cellular material for cytology. The procedure is best done with an experienced cytopathologist present to analyse the sample by microscopy and determine on site whether there is adequate and representation cells in the aspirated to make the diagnosis. Inadequate tissue sampling is one the greatest drawbacks of this technique.

Core needle biopsy has an advantage over FNA because it uses a larger diameter needle allowing for a more adequate tissue sample to be obtained. However, unlike FNAs, the CNB may be done blindly and so you may not have any indication of the tumour content until after the sample has been processed and examined microscopically. Depending on the location of the tumour, it may be possible to safely obtain multiple CNB samples. The larger sample size provides more ample tumour tissue to make a histologic diagnosis of non-small cell lung cancer and perform biomarker testing. Core needle biopsy complications will depend on a number of factors including the organ that is being biopsied. Compared with FNA, core needle biopsy does not appear to result in a higher complication rates for hemoptysis (coughing up blood) or pneumothorax (air in the pleural space).[7]

The extent or stage of the lung cancer at diagnosis will often determine the choice of procedures used for obtaining tumour tissue to determine a histologic diagnosis and test for biomarkers. In patients with cancer localized to the lung without involvement of the lymph glands in the centre of the chest (mediastinum) or pleural space, their disease may be classified as stage I or II and therefore potentially curable by resection of the primary tumour. If it is very likely that the patient is a candidate for a curative lung resection, then a FNA may be the preferred technique for obtaining a diagnosis of NSCLC. The amount of tumour available to the pathologist after a lung cancer resection will be more than adequate for biomarker testing.

The approach may be different for patients with inoperable lung cancer, where cure by surgery is not thought to be possible. Since testing for the EGFR and ALK mutations is recommended in advanced stage lung cancer, performing a CNB is more likely to yield sufficient quantities of representative tumour tissue to confirm the diagnosis of NSCLC, subclassify the tumour and have sufficient tissue remaining for molecular diagnostic studies.

Compared to CNB, the interpretation of FNA specimens or exfoliation cytology is limited by the smaller sample size, a sampling error or the lack of a histologic pattern. The size of a biopsy is important, but the sample must obviously be representative of the tumour. For molecular testing, the preference between FNA versus CNB will vary between laboratories but as a general rule specimens with a small amount of tumour but a high tumour cellularity may be more appropriate compared with a larger biopsy with a low cellularity.

It is very important that the respirologists and thoracic surgeons communicate effectively with their radiologist colleagues when ordering image guided biopsies to help determine the best technique to obtain a diagnosis and have ample tissue left over for molecular testing.

The Role of the Pathologist

Regardless of the method used to acquire tumour tissues samples, the specimens will need to be processed in such a way that will allow the pathologist to view the tumours cells and confirm the diagnosis. Cellular material obtained by FNA can be spread onto glass slides for examination by microscopy by the pathologist. Residual material acquired from FNA can be concentrated by centrifugation of the cellular solution to make a pellet, which is then fixed in a formalin solution and embedding in paraffin. Slides made from the paraffin blocks are useful adjuncts for establishing a diagnosis using immunocytochemistry and for molecular testing. For bronchoscopic forceps biopsies and CNBs, the samples are suspended in formalin, then embedded in a

Communication between histo-pathologists, cytologists and laboratory technicians is critical.

paraffin, from which thin slices can be cut to make slides for routine microscopy and immunohistochemistry (IHC). The distinction between subtypes of NSCLC can be made by morphology alone provided there is adequate tissue. In cases where there is no clear differentiation by microscopy, IHC markers, such as the adenocarcinoma marker TTF-1 and the squamous marker P63, can be very helpful in distinguishing subtypes.

An excessive number of slides made from the tumour block for histology and additional IHC markers will limit the amount of sample that can be used for biomarker detection. Communication between histo-pathologists, cytologists and laboratory technicians is critical in preserving biopsy tissue for subsequent molecular testing.

Slides with sufficient tumour cells can later be used for biomarker detection using IHC to detect the ALK fusion protein, or fluorescence in situ hybridization (FISH) to determine the presence of an ALK fusion gene. Although both these assays can be used independently to detect ALK fusions, many labs will perform reflex confirmation of a positive IHC result with FISH.

EGFR mutational analysis and ALK testing are often done sequentially, first by extracting a small amount of genetic material from formalin fixed paraffin embedded (FFPE) biopsy tissue for EGFR testing, and then making slides for ALK fusion gene detection. However, in some cases where there is insufficient tissue in the biopsy sample, the tumour may be exhausted from the paraffin block precluding the possibility of further biomarker testing. A quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR) method has been successfully used to detect the presence of ALK fusions in cases of low tumour content in biopsy samples.[8,9] Unlike early allele-specific RT-PCR assays, which can only detect specific ALK fusion RNA expression, newer PCR assays are able to detect any ALK oncogenic fusion transcript and upregulation of the gene.

Analytical methods that test for the presence of multiple, different cancer biomarkers simultaneously will likely replace many single biomarker assays.

Even when biopsy samples are deemed generous, there are a number of factors that can adversely affect the quality of the biomarker nucleic acids and protein hampering their detection. The handling and processing of the tissue biopsy in the bronchoscopy suite, radiology department and operating room is very important. Significant degradation of nucleic acid can occur before the sample is suspended in fixative, so specimens should be fixed in formalin within a pre-specified time of the biopsy for a maximum of 6 to 48 hours. Fixation in formalin beyond 48 hours may modify nucleic acids making biomarker testing difficult to impossible to complete.[10] These times will vary between institutions.

The preservation of the quality of biopsy samples, which have been embedded in paraffin blocks, is very important because archival specimens are frequently used in biomarker testing. Positive EGFR mutation and ALK fusion results have been obtained from specimens acquired five to 10 years earlier. Tumour blocks should be stored without cut surfaces to prevent damage caused by oxidation, light and water exposure, and infestation.

Specialists agreed that acquiring a sufficient quantity of quality tissue remains a challenge in many cases.

The Role of the Multidisciplinary Team

A recent industry sponsored survey has revealed some differing views between lung specialists and pathologists regarding the most appropriate biopsy methods for acquiring sufficient lung cancer tissue samples to test for biomarkers. Both groups of specialists agreed that acquiring a sufficient quantity of quality tissue remains a challenge in many cases. Where the specialists differed in their opinions was in their preferences for either FNA versus CNB for the best method for obtaining tissue for biomarker studies.

The survey also supported the role of the multidisciplinary team (MDT) in lung cancer care, with the majority of specialists reporting that they frequently consulted with their medical and radiation oncology colleagues either informally or as participants in multidisciplinary lung cancer tumour boards or conferences.[11] The role of the oncology MDT in tumour molecular profiling will continue to be relevant as more clinically relevant actionable genetic mutations are discovered and corresponding companion assays and targeted treatments are developed.[12]

It is important that cancer centres, pathology departments and molecular diagnostic laboratories develop effective communication strategies and standard operating procedures (SOPS) for the biomarker testing and reporting of results. Ideally, there should be designated clerical members in cancer centres and pathology departments who coordinate the requisitioning for biomarker testing and transportation of tumour blocks between pathology departments and molecular diagnostic laboratories.

Recommendations

1. **Canadian cancer centres, pathology departments and molecular diagnostic laboratories should collaborate in the development and implementation of clear strategies for biomarker testing.**
2. **Physicians involved in the acquisition and processing of tumour biopsies for biomarker testing should be members of multidisciplinary teams.**
3. **The optimum biopsy technique for obtaining**

an adequate tumour tissue sample for diagnosis and biomarker testing should be discussed within multidisciplinary teams.

4. **Pathology laboratories should have standard operating procedures for the processing, storage, and transportation of tumour samples that may be tested for biomarker.**

David Saltman MD, PhD, FRCPC is a Medical Oncologist in Victoria, BC and a Director of the CACC.

References

1. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 2010;362: 2380–2388.
2. Shaw A, Kim DW, Nagagawa K, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. N Engl J Med. 2013;368(25):2385-2394.
3. Thunnissen E, Kerr KM, Herth FJ, et al. The challenge of NSCLC diagnosis and predictive analysis on small samples. Practical approach of a working group. Lung Cancer. 2012;76(1):1-18.
4. Gu P, Zhao YZ, Jiang LY, Zhang W, Xin Y, Han BH. Endobronchial ultrasound-guided transbronchial needle aspiration for staging of lung cancer: a systematic review and meta-analysis. European Journal of Cancer. 2009;45:1389–1396. [Review]
5. Uskul BT, Turker H, Gokce M, Kant A, Arslan S, Turan FE. CT-guided transthoracic fine needle aspiration of pulmonary lesions: accuracy and complications in 134 cases. Tuberkuloz ve Toraks. 2009;57:177–185.
6. Priola AM. Accuracy of CT-guided transthoracic needle biopsy of lung lesions: Factors affecting diagnostic yield. Radiologia Medica. 2007;112:1142–1159.
7. Gong Y. Transthoracic fine-needle aspiration vs concurrent core needle biopsy in diagnosis of intrathoracic lesions: A retrospective comparison of diagnostic accuracy. American Journal of Clinical Pathology. 2006;125:438–444.
8. Wang R, Pan Y, Li C, et al. The use of quantitative real-time reverse transcriptase PCR for 5' and 3' portions of ALK transcripts to detect in lung cancer. Clin Cancer Res 2012;18:4725-4732.
9. Gruber K, Horn H, Kalla J, et al. Detection of rearrangements and transcriptional up-regulation of ALK in FFPE lung cancer specimens using a novel, sensitive, quantitative reverse transcription polymerase chain reaction assay. J Thorac Oncol 2014; 9(3): 307-315.
10. Fowler DL, Cunningham BR, Mason JT, et al. The Effect of Formaldehyde Fixation on RNA: Optimization of Formaldehyde Adduct Removal. J Mol Diagn. May 2011; 13(3): 282–288.
11. http://us.boehringer-ingelheim.com/news_events/press_releases/press_release_archive/2013/05-07-13-surveys-medical-community-biomarker-testing-lung-cancer.html
12. Blackhall F, Thatcher N, Bouton R, et al. The impact on the multidisciplinary team of molecular profiling for personalized therapy in non-small cell lung cancer. Lung Cancer 2013;79(2):101-103.

EPIGENOME RESEARCH IN BRITISH COLUMBIA[1]

The BC Cancer Agency was part of a seven year project mapping the epigenome. The term "genome" refers to all the DNA within a cell, and the term "epigenome" refers to the chemical modifications of DNA and proteins that control the structure and activity of the genome. Epigenomes either cause the genome to stay healthy or develop diseases, such as cancer, because they produce the code for cellular properties that distinguish one cell type from another. A better understanding of the epigenome may assist in the design of new treatments. The project, called The National Institutes of Health (NIH) Roadmap Epigenomics Program, provides a core set of data, methodology and infrastructure for studying the role of the epigenome in human health and disease. The original goal was to map 25 normal reference epigenomes, but new technology allowed the team to produce 111 highly detailed maps on how the epigenome varies and operates in different settings.

The Roadmap Epigenomics Program was the first large-scale epigenome mapping initiative in the world, and has inspired similar mapping efforts, which are united by the International Human Epigenome Consortium (IHEC). The IHEC aims to coordinate the production of at least 1,000 human epigenome maps.

Just as the Human Genome Project provided a map of the genes of the human genome, the Roadmap Epigenomics Program offers a resource for understanding how our genetic blueprint is interpreted in different cell and tissue types. The next step will be to map the epigenetic profiles of individuals to understand more about how they vary from person to person and to establish causes between any of these "epigenomic marks" and disease.

IHEC encompasses the Canadian Epigenetic and Environment and Health Research Consortium (CEEHRC), and aims to coordinate the production of at least 1,000 human epigenome maps. All IHEC data is available for use by researchers from around the world, with the ultimate aim of improving human health through a better understanding of disease prevention and potential therapeutic options.

1. BC Cancer Agency website: http://www.bccancer.bc.ca/NR/rdonlyres/B9CBD0FE-6A2D-4755-AA6C-32C4571CFBE5/74098/02132015_BCCA_NR_NaturepaperepigenomicsFinal2.pdf