## EDITORIAL: CELL AND MOLECULAR BIOLOGY

# Lung cancer: multidisciplinary approach for management

**Cell and Molecular Biology Assembly contribution to the celebration of 20 years of the ERS**

E. Brambilla

The Cell and Molecular Biology Assembly, in association with the Thoracic Oncology Assembly, has been leading a Task Force on an adenocarcinoma multidisciplinary classification, which includes histomolecular and radiological correlation as well as oncology and surgical management. With >1.3 million deaths annually [1, 2], lung cancer is the leading cause of cancer death worldwide [3, 4]. The dismal prognosis of lung cancer is due to lack of early diagnostic tools, presentation at a late stage and an at best modest effect of chemotherapy. The major cause of lung cancer is tobacco smoking (85% of lung cancer). Major requirements in the fight against lung cancer can be met by tobacco smoking cessation, and by understanding the genetic and epigenetic origins of progression of the disease, since smokers remain at risk 15 yrs after cessation. In addition, recent epidemiological data point to a preoccupying increase in incidence of lung adenocarcinoma in never-smokers, particularly in females. Adenocarcinoma is the most common histological subtype of lung cancer in most countries, accounting for almost half of all lung cancer. Within lung adenocarcinoma, a widely divergent clinical, radiological, molecular and pathologic spectrum exists. In the past decade and despite spectacular advances in understanding of the molecular origins of lung cancer, there has remained a need for universally accepted criteria for adenocarcinoma histological subtypes and their histomolecular correlations.

### ADENOCARCINOMA MULTIDISCIPLINARY CLASSIFICATION

The 2004 World Health Organization classification [5] has not been used on a widespread level among pathologists and medical oncologists, and while enormous resources are being spent on trials evaluating molecular and therapeutic aspects of adenocarcinoma, the development of standardised criteria for classification are of great importance to the advancement of the impact of research and the improvement of patient care. In their wide view of the lung cancer human problem and challenge, the International Association for the Study of Lung Cancer (IASLC) and the two largest respiratory societies, the European Respiratory Society (ERS) and the American Thoracic Society (ATS), support a collaborative effort for an international multidisciplinary classification of lung adenocarcinoma in order to provide an integrated clinical, radiological,

molecular and pathologic approach to classification, with the goal of identifying prognostic and predictive factors as well as therapeutic targets. This is the objective of the Task Force of the ATS/ERS, which has been ongoing from 2007 to 2010 (unpublished study; personal communication: W.D. Travis, MSKCC, New York, NY, USA, and E. Brambilla). Overall, histopathology is the backbone of this classification but lung cancer diagnosis is a multidisciplinary process.

The ATS/ERS multidisciplinary panel performed a systematic review of the literature including all features of clinical, pathologic, radiologic, molecular and surgical disciplinaries.

The different histological categories of the proposed classification are subdivided into preinvasive lesions, minimally invasive adenocarcinoma and invasive adenocarcinoma (table 1).

The multidisciplinary classification will not only propose the guidelines for classification based on surgical resection material but will address the classification problem for small biopsies and cytology. Indeed, targeted therapies are indicated in patients with advanced disease and in 70% of the cases, lung cancer is diagnosed and staged by small biopsies or cytology, with increasing use of transbronchial needle aspiration (TBNA), endobronchial ultrasound-guided TBNA and oesophageal ultrasound. When only cytological or small biopsy samples are available, most pathologists are able to correctly distinguish small cell lung cancer from nonsmall cell lung cancer. However, targeted and customised therapy requires a choice between squamous cell carcinoma *versus* adenocarcinoma, which is a specific diagnosis problem because tumour heterogeneity, poor differentiation, presence of artefacts limit the diagnostic yield in the small biopsies.

The ERS/ATS Task Force will propose a pathologic diagnosis algorithm for the majority of lung cancer patients who present with locally advanced or metastatic disease. Due to the need for clear separation of squamous cell carcinoma from adenocarcinoma (severe haemorrhage under treatment with bevacizumab in squamous cell carcinoma [6]; efficacy of pemetrexed restricted to adenocarcinoma and large cell carcinoma [7]), a strategic use of small biopsies and cytology samples is important in order to use the minimum specimen necessary for an accurate diagnosis, while preserving as much tissue as possible for potential molecular study. For any specimen showing nonsmall cell lung carcinoma lacking morphological evidence of either definite squamous or adenocarcinomatous differentiate, immunohistochemistry will be used to refine the

CORRESPONDENCE: E. Brambilla, CHU Albert Michallon, Dept of Pathology, INSERM U823, University Joseph Fourier, Grenoble, France. E-mail: ebrambilla@chu-grenoble.fr

| **TABLE 1** | Tentative proposals of the International Association for the Study of Lung Cancer (IASLC)/ American Thoracic Society (ATS)/European Respiratory Society (ERS) for the classification of lung adenocarcinoma |
|---|---|

**Preinvasive lesions**

Atypical adenomatous hyperplasia

Adenocarcinoma in situ (formerly BAC)

   Nonmucinous

   Mucinous (very rare)

**Minimally invasive adenocarcinoma**

A lepidic predominant tumour with ⩽5 mm or >10% invasion

**Invasive adenocarcinoma**

Lepidic predominant (formerly non-mucinous

   bronchioalveolar carcinoma pattern)

Acinar predominant

Papillary predominant

Micropapillary predominant

Solid predominant

**Variants**

Mucinous adenocarcinoma with lepidic pattern

   (formerly mucinous bronchioalveolar carcinoma)

Mucinous cystadenocarcinoma

Colloid

Fetal (low and high grade)

Enteric

diagnosis. TTF1 appears to be the best marker for adenocarcinoma with mucine production, as well P63 and cytokeratin 5/6, which are considered reliable markers for squamous histology (unpublished study; personal communication: K. Kerr, Aberdeen Royal Infirmary, Aberdeen, UK) [6, 8–14]. This will cover ~20% of cases referred to as nonsmall cell lung carcinoma (none otherwise specified) with routine histology and immunohistochemical expression. These cases will be processed to molecular analysis (epidermal growth factor receptor (EGFR), Ras, EML4-ALK (echinoderm microtubule-associated protein-like 4 gene and anaplastic lymphoma kinase gene) fusion) along with adenocarcinoma in order to determine targeted treatment indication. It is clear that large cell carcinoma should not be diagnosed on small biopsies or cytology specimens: this is a diagnosis reserved for surgical samples. The strategic use of molecular markers is rewarding for small biopsies since assessment of EGFR and KRAS mutation help in selecting patients to be treated with EGFR tyrosine kinase inhibitor agents. Some gene expression of markers of sensitivity to specific cytotoxic agents, such as ERCC1, BRCA1 for platinum, RRM1 for gemcitabine, or thymidilate-synthase for antifolates (pemetrexed) is recommended [15, 16].

## NEW TARGETED THERAPIES

The Iressa Pan-Asia Study (IPASS) demonstrated that patients with EGFR mutation have a longer time to progression when treated with gefitinib than with conventional cytotoxic chemotherapy [17]. This allowed Food and Drug Administration approval of EGFR inhibitor gefitinib as first-line therapy in advanced lung adenocarcinoma in the USA and most European countries. Recently, a new predictive biomarker has been

identified in patients with nonsmall cell lung carcinoma, the EML4-ALK fusion. On chromosome 2, inversion within chromosome 2p and small deletion at the 5′ end of EML4 give rise to a transforming fusion, EML4-ALK, which activates the kinase domain of ALK, leading to an oncogenic constitutive activation of ALK activity [18–20]. Preliminary data from a small series of patients with EML4-ALK fusion treated by an inhibitor of MET and ALK demonstrated a 50% response rate with tumour shrinkage [21]. The EML4-ALK fusion gene is mutually exclusive with EGFR and/or KRAS mutation and should be searched for in EGFR, KRAS-negative cases in nonsmokers or never-smokers.

## SIGNALLING PATHWAYS IN LUNG ADENOCARCINOMA

New documentation of the histogenetic origin of lung adenocarcinoma subtypes and molecular features has increased, which has important implications in lung adenocarcinoma patients, with new signalling pathways serving as a roadmap for new targeted therapies [22]. Regarding the histogenetic origins of lung adenocarcinoma subtypes, generation of the two embryologic lung buds at anterior oesophagus and branching results in two compartments: the central compartment of conducting airways; and the subsequent development of the terminal sac and alveoli forming the distal compartment identified by TTF1 expression, a differentiation gene, and a potentially lineage-specific survival oncogene in some lung adenocarcinoma [23–25]. The central compartment gives rise to tumours that are TTF1 negative with candidate progenitor cells being the bronchial basal cells and the mucus cells. The progenitor cell Clara type II pneumocyte peripheral compartment has two leading signalling pathways, one activated by EGFR mutation in nonsmokers, the other one by KRAS mutation in smokers, the choice of which is essentially dictated by smoking status. However, only 15–30% of the patients have one of those mutations that is completely mutually exclusive. These mutations guide the therapy since EGFR has been shown to be the specific target for therapy by tyrosine kinase/EGFR inhibitors according to the IPASS trial [17], whereas KRAS mutation is now considered as a target for MEK inhibitors. Both gefitinib and MEK inhibitors have been approved as first- or second-line therapy in advanced patients with the required mutation.

Lung cancer with ALK translocations is now the target of a specific ALK inhibitor, EML4-ALK. Epidemiological characteristics of this adenocarcinoma type defined by a genetic trait include: a prevalence of 5% of lung adenocarcinoma; a variety of typical histological features, including acinar, solid and cribriform patterns; signet-ring cells with or without mucin production. This is another histomolecular feature that is providing a potential targeted therapy since these patients are exquisitely sensitive to ALK and MET inhibitor (PF230411066).

Wide genomic gene expression profiling has shown an exquisite fit between histological subclassification as newly proposed and gene expression clusters and pathways: a large multicentre blinded evaluation of eight independently derived genomic signatures of prognosis was driven by the American Director challenge, distinguishing three clusters of genes with prognostic relevance, one of which is enriched with cell proliferation genes [26]. Validation has been performed on external platforms of the prognostic value of the three clusters

(unpublished studies; Y. Yatabe, Aichi Cancer Center Hospital, Nagoya, Japan, and E. Brambilla).

## GENOMICS AND EPIGENOMICS IN LUNG CANCER

Recently, the molecular landscape of lung adenocarcinoma has been reshaped by high throughput analysis of DNA mutations [27]. DNA sequencing of 623 known cancer-related genes in 188 adenocarcinoma allowed the description of 1,013 somatic mutations, 580 of which have biological relevance. In addition to confirmation of *bona fide* tumour suppressor genes affected by deletion or nonsense mutation P53, P16$^{INK4}$, STK11/LKB1, new mutations of supressor genes, such as NF1 and RB1, were described with a frequency of 10% each. STK11/LKB1 mutation controlling the AMPK-mTOR pathway appears as the third in frequency in adenocarcinoma, thus filling the ''unknown'' gap beside EGFR or Ras mutations. Oncogenes characterised by activating mutations were enlighted, in addition to known EGFR and Ras mutations: ERBB4, EPHA3, KDR, FGFR4, all of which contain tyrosine-kinase domains targetable by tyrosine-kinase inhibition therapy. Other mutual exclusions were shown in addition to EGFR KRAS, such as KRAS/STK11 *versus* NF1 and P53 *versus* ATM of their common functional pathway on DNA damage-induced G2 cell cycle arrest.

Gene copy number alterations have been fully examined by SNP arrays and correlate with signalling pathway [24]. A number of mutations and gene copy number alterations target key functional pathways containing both oncogene and tumour suppressor genes. Strong aggregations of these alterations alter the function of tyrosine-kinase pathways and cell cycle (CDKN2/P53/RB1) pathways.

Gene expression profiling now shows a direct relationship of adenocarcinoma heterogeneity with different expression of cancer-related pathways. Gene clustering and pathways mostly recapitulate adenocarcinoma histological patterns and subtypes [28]. They may better predict clinical outcome and pathway deregulation signature than pathologic pattern itself and will also allow prediction of sensitivity to therapeutic agents targeting their components [29–31].

It has recently appeared that the epigenetic landscape of cancer cells is profoundly distorted. Epigenetic modifications are defined as mechanisms of gene expression regulation which do not change the primary DNA sequence. They include DNA methylation, covalent post-translational modification of histone proteins, and RNA-mediated gene silencing (microRNA). Furthermore, it is now apparent that epigenetic modifications contribute to lung tumour formation. This involves both global genomic hypomethylation, leading to chromosomal instability, and regional hypermethylation of DNA at promoter regions of tumour suppressor genes [32]. By this last mechanism of silencing, the expression of tumour suppressor genes in the cancer cell can be reduced or eliminated as an alternative mechanism to genetic mutation and allelic loss. Indeed, nucleosomal histone proteins are no longer considered to be simple ''DNA packaging proteins''. They are recognised as dynamic regulators of gene activity that undergo many post-translational chemical modifications on specific amino-acid residues of core histone tails, including acetylation, methylation, phosphorylation, ubiquitinilation and sumoylation [33]. Specific combinations of histone modifications confer the overall expression status of a region of chromatin, a theory known as the ''histone code'' hypothesis. In this regard, histone covalent modification influences a multitude of cellular process like development, cell differentiation, response to environmental signals, and tumourigenesis. Histone H4 is one of the nucleosomal core histones in which acetylation and methylation at specific lysine in the N terminal tails affect higher order chromatin structure, transcriptional regulation and DNA repair. We have shown their role in the prognosis of small T1N0 adenocarcinoma and early events in squamous cell carcinoma [34].

RNA-mediated gene silencing involving microRNA is a promising diagnostic and therapeutic target in lung cancer. MicroRNAs are small noncoding RNAs, encoded in fragile genome sites, subjected to amplification, deletion or silencing by methylation. There are two families of microRNA concerning tumourigenesis: those with tumour suppressor gene functions, which are mostly inactivated by deletion, methylation, or mutation; and others with oncogenic functions, which are amplified on amplicons or transcriptional targets of oncogenes. In 2005, the first identification of the connection between microRNA expression and genes responsible for pulmonary carcinogenesis was provided [35]. An inverse relationship was found between the microRNA let-7 and activation of Ras protein: this microRNA was encoded on fragile genome site 3p, deleted in 90% of squamous cell carcinomas. Furthermore, YANAIHARA *et al.* [36] demonstrated five microRNAs with prognostic value in patients with pulmonary adenocarcinoma. The microRNA profile in lung cancer is found to be completely specific of normal *versus* tumours and of lung cancer *versus* any other cancer. One of them, microRNA205, allowed the diagnosis of epidermoid carcinoma with 98% specificity *versus* adenocarcinoma [37]. Since microRNA circulates intact in the serum, these are promising early detection markers and prognostic tools. The potential of antagonism of miRNA using anti-microRNA complementary compounds is extremely promising.

## CONCLUSION

The new multidisciplinary classification of adenocarcinoma will provide clinical significance to the histologic subtyping and is correlated with prognosis and molecular signalling pathways. High throughput analysis of genomic and epigenomic alteration will include, in the next year, a full description of DNA methylation changes, discovery of a new generation of genes epigenetically deregulated responsible for drug resistance, and potential targets for therapy. Genome-wide promoter epigenetic methylation and histone modification, and microRNA expression profiles of well-defined histological types of lung cancer are promising in identification of genetic and epigenetic alterations that account for carcinogenesis, primary and secondary resistant to targeted treatment.

## STATEMENT OF INTEREST

A statement of interest for E. Brambilla can be found at www.erj.ersjournals.com/misc/statements.dtl

## REFERENCES

1 Samet J, Avila-Tang E, Boffeta P, *et al.* Lung cancer in never smokers: clinical epidemiology and environmental risk factors. *Clin Cancer Res* 2009; 15: 5626–5645.

2  Rudin CM, Avila-Tang E, Harris C, *et al.* Lung cancer in never smokers: molecular profiles and therapeutic implications. *Clin Caner Res* 2009; 15: 5646–5661.

3  Boyle P, Levin B. World Cancer Report. Lyon, International Agency for Research on Cancer, 2008.

4  Parkin DM, Bray F, Ferlay J, *et al.* Global cancer statistics, 2002. *CA Cancer J Clin* 2005; 3: 74–108.

5  Johnson DH, Fehrenbacher L, Novotny WF, *et al.* Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. *J Clin Oncol* 2004; 22: 2184–2191.

6  Scagliotti GV, Parikh P, von Pawel J, *et al.* Phase III study comparing cisplatin plus gemcitabine with cisplatin plus peme-trexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. *J Clin Oncol* 2008; 26: 3543–3551.

7  Wu M, Wang B, Gil J, *et al.* p63 and TTF-1 immunostaining. A useful marker panel for distinguishing small cell carcinoma of lung from poorly differentiated squamous cell carcinoma of lung. *Am J Clin Pathol* 2003; 119: 696–702.

8  Chu PG, Weiss LM. Expression of cytokeratin 5/6 in epithelial neoplasms: an immunohistochemical study of 509 cases. *Mod Pathol* 2002; 15: 6–10.

9  Kargi A, Gurel D, Tuna B. The diagnostic value of TTF-1, CK 5/6, and p63 immunostaining in classification of lung carcinomas. *Appl Immunohistochem Mol Morphol* 2007; 15: 415–420.

10  Khayyata S, Yun S, Pasha T, *et al.* Value of P63 and CK5/6 in distinguishing squamous cell carcinoma from adenocarcinoma in lung fine-needle aspiration specimens. *Diagn Cytopathol* 2009; 37: 178–183.

11  Ou SH, Zell JA. Carcinoma NOS is a common histologic diagnosis and is increasing in proportion among non-small cell lung cancer histologies. *J Thorac Oncol* 2009; [Epub ahead of print PMID: 19701111].

12  Scagliotti GV, Parikh P, von Pawel J, *et al.* Phase III study comparing cisplatin plus gemcitabine with cisplatin plus peme-trexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. *J Clin Oncol* 2008; 26: 3543–3551.

13  Loo PS, Thomas SC, Nicolson MC, *et al.* Subtyping of undiffer-entiated non-small cell carcinomas in bronchial biopsy specimens. *J Thorac Oncol* 2010; (In press).

14  Nicholson AG, Gonzalez D, Shah P, *et al.* Refining the diagnosis and EGFR status of non-small cell carcinoma in biopsy and cytologic material, using a panel of mucin staining, TTF-1, cytokeratin 5/6, and P63, and EGFR mutation analysis. *J Thorac Oncol* 2010; [Epub ahead of print PMID: 20068475].

15  Bepler G, Kusmartseva I, Sharma S, *et al.* RRM1 modulated *in vitro* and *in vivo* efficacy of gemcitabine and platinum in non-small-cell lung cancer. *J Clin Oncol* 2006; 24: 4731–4737.

16  Olaussen KA, Dunant A, Fouret P, *et al.* DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemo-therapy. *N Engl J Med* 2006; 355: 983–991.

17  Mok TS, Wu YL, Thongprasert S, *et al.* Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* 2009; 361: 947–957.

18  Shaw AT, Yeap BY, Mino-Kenudson M, *et al.* Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. *J Clin Oncol* 2009; 27: 4247–4253.

19  Wong DW, Leung EL, So KK, *et al.* The EML4-ALK fusion gene is involved in various histologic types of lung cancers from nonsmokers with wild-type EGFR and KRAS. *Cancer* 2009; 115: 1723–1733.

20  Soda M, Choi YL, Enomoto M, *et al.* Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* 2007; 448: 561–566.

21  Kwak EL, Camidge DR, Clark J, *et al.* Clinical activity observed in a phase I dose escalation trial of an oral c-met and ALK inhibitor, PF-02341066. *J Clin Oncol* 2009; pp. 3509.

22  Brambilla E, Gazdar A. Pathogenesis of lung cancer signalling pathways: roadmap for therapies. *Eur Respir J* 2009; 33: 1485–1497.

23  Kim CF, Jackson EL, Woolfenden AE, *et al.* Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell* 2005; 121: 823–835.

24  Weir BA, Woo MS, Getz G, *et al.* Characterizing the cancer genome in lung adenocarcinoma. *Nature* 2007; 450: 893–898.

25  Tanaka H, Yanagisawa K, Shinjo K, *et al.* Lineage-specific dependency of lung adenocarcinomas on the lung development regulator TTF-1. *Cancer Res* 2007; 67: 6007–6011.

26  Shedden K, Taylor JM, Enkemann SA, *et al.* Gene expression-based survival prediction in lung adenocarcinoma: a multi-site, blinded validation study. *Nat Med* 2008; 14: 822–827.

27  Ding L, Getz G, Wheeler DA, *et al.* Somatic mutations affect key pathways in lung adenocarcinoma. *Nature* 2008; 455: 1069–1075.

28  Motoi N, Szoke J, Riely GJ, *et al.* Lung adenocarcinoma: modification of the 2004 WHO mixed subtype to include the major histologic subtype suggests correlations between papillary and micropapillary adenocarcinoma subtypes, EGFR mutations and gene expression analysis. *Am J Surg Pathol* 2008; 32: 810–827.

29  Bild AH, Yao G, Chang JT, *et al.* Oncogenic pathway signatures in human cancers as a guide to targeted therapies. *Nature* 2006; 439: 353–357.

30  Sos ML, Michel K, Zander T, *et al.* Predicting drug susceptibility of non-small cell lung cancers based on genetic lesions. *J Clin Invest* 2009; 119: 1727–1740.

31  Beer DG, Kardia SL, Huang CC, *et al.* Gene-expression profiles predict survival of patients with lung adenocarcinoma. *Nat Med* 2002; 8: 816–824.

32  Shames DS, Girard L, Gao B, *et al.* A genome-wide screen for promoter methylation in lung cancer identifies novel methylation markers for multiple malignancies. *PLoS Med* 2006; 3: e486.

33  Esteller M. Cancer epigenomics: DNA methylomes and histone-modification maps. *Nat Rev Genet* 2007; 8: 286–298.

34  Van Den Broeck A, Brambilla E, Moro-Sibilot D, *et al.* Loss of histone H4K20 trimethylation occurs in preneoplasia and influ-ences prognosis of non-small cell lung cancer. *Clin Cancer Res* 2008; 14: 7237–7245.

35  Johnson SM, Grosshans H, Shingara J, *et al.* RAS is regulated by the let-7 microRNA family. *Cell* 2005; 120: 635–647.

36  Yanaihara N, Caplen N, Bowman E, *et al.* Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell* 2006; 9: 189–198.

37  Bishop JA, Benjamin H, Cholakh H, *et al.* Accurate classification of non-small cell lung carcinoma using a novel microRNA-based approach. *Clin Cancer Res* 2010; 16: 610–619.