The biomolecular era for thoracic surgeons: the example of the ESTS Biology Club

Isabelle Opitz¹, Thomas A. D’Amico², Gaetano Rocco³

¹Division of Thoracic Surgery, University Hospital Zurich, Switzerland; ²Division of Thoracic Surgery, Department of Surgery, Duke University Medical Center, Durham, USA; ³Department of Thoracic Surgery and Oncology, Istituto Nazionale Tumori, Fondazione Pascale, IRCCS, Naples, Italy

Abstract: Understanding basic mechanisms of lung disease may help to move forward the management of our patients. Molecular biology has affected our diagnostic and therapeutic pathways in the direction of personalized medicine not only for thoracic malignancies. Accordingly, thoracic surgeons are becoming increasingly aware that specific knowledge of genetic and epigenetic alterations may influence their clinical behavior—from the ward to the operating room (OR). In this continuously evolving scenario, surgical societies have perceived the increasing relevance of biomolecular medicine in the practice of modern thoracic surgery. More recently, in the spirit of mutual collaboration between sister societies, the European Society of Thoracic Surgeons (ESTS) has adopted the concept of the American Association for Thoracic Surgery (AATS) incorporating one session dedicated to the Biology Club within the Annual Meeting Program. The aim of the ESTS Biology Club is to outline and sponsor the new profile of the surgeon scientist during the only world meeting exclusively focused on general thoracic surgery. The following article will summarize the significance of this and give an update on molecular biology tools for thoracic malignancies.

Keywords: Molecular biology; thoracic surgery; non-small cell lung cancer (NSCLC); mesothelioma; the European Society of Thoracic Surgeons (ESTS)

Submitted May 02, 2014. Accepted for publication May 05, 2014.
doi: 10.3978/j.issn.2072-1439.2014.05.01

View this article at: http://www.jthoracdis.com/article/view/2489/3050

Introduction—the new era

Would it be possible today for thoracic surgeons to ignore the information resulting from the so-called “oncogenic addiction”—at least the EGFR mutation or the anaplastic lymphoma kinase (ALK) translocation in lung cancer? How much has the biomolecular era affected our diagnostic and therapeutic pathways when dealing with thoracic cancers? Thoracic surgeons would concur on the statement that their specialty has changed dramatically since the introduction of minimally invasive techniques facilitated by the refinement of anesthetic techniques, the new thresholds for surgery-related risk assessment, the implementation of quality metrics to assess performance, and the need to comply with cost-effectiveness criteria while running a surgical service (1).

The multidisciplinary approach to thoracic oncology based on the integration of competences is gaining an unprecedented momentum from the experience of lung cancer screening teams. In these groups, surgeons have a fundamental role in interpreting low-dose screening CT (LDCT) and suggesting the most appropriate diagnostic procedure for the screen-detected nodules (2). It is not by chance that the experience from a lung cancer screening program led by a surgeon has contributed to a five-fold reduction of the false positive results by adding the assessment of micro RNA (miRNA) signatures to LDCT (3). Accordingly, thoracic surgeons are becoming increasingly aware that specific knowledge of genetic and epigenetic alterations may influence their clinical behavior—from the ward to the operating room (OR) (1). In this setting,
it would be difficult to argue against the benefit on patient management of the recently introduced new classification of adenocarcinoma where the integration of pathological, biomolecular, radiological, and clinical features is crucial to the prediction of prognostic correlates of adenocarcinoma subtypes (4).

The future will depend on initiatives such as the Lungscape project from the European Thoracic Oncology Platform—a network of cancer centres (http://www.etop-eu.org)—providing an innovative platform to conduct biomolecular investigations into the nature and outcome of non-small cell lung cancer (NSCLC) and with Mesoscape of malignant pleural mesothelioma (MPM). The aims are to harmonize standards and improve the quality of genetic testing in cancer centres, and increase the knowledge about latest targeted therapies; both initiatives where thoracic surgeon are and will be further involved in the future. Moreover, the fundamental work by Pass and colleagues on fibulin 3 as a new biomarker for mesothelioma (5) has conclusively outlined the role of the surgeon scientist in modern medicine.

The new profile of the surgeon scientist indeed represents a cultural revolution in thoracic surgery. Excellence in the OR, in the intensive care unit and on the ward is coupled with an unprecedented attention to clinical and basic research which translates in focused knowledge of the biomolecular changes causing thoracic diseases. Concomitantly, a new measurement of performance has been inherited from the world of basic researchers and progressively popularized among academic surgeons. In spite of undisputed flaws and limitations, the widely accepted bibliometric parameters (i.e., the impact factor and the h-index) have become, especially in Europe, important criteria to define career progress and actual leadership in academic institutions (6). In this continuously evolving scenario, surgical societies have perceived the increasing relevance of biomolecular medicine in the practice of modern thoracic surgery. The American Association for Thoracic Surgery (AATS) has supported for years an ancillary event to the AATS Annual Meeting focused on the biology of thoracic conditions of surgical interest. The AATS General Thoracic Biology Club has gathered an ever increasing membership attracted by a program including presentations of experimental evidence of biomolecular pathways studied at highly respected international academic institutions. It is notable that the surgeons that have presented at this meeting have developed a successful research portfolio and have achieved important leadership positions.

More recently, in the spirit of mutual collaboration between sister societies, the European Society of Thoracic Surgeons (ESTS) has adopted this concept, incorporating one session dedicated to the Biology Club within the Annual Meeting Program, with the aim of outlining and sponsoring the new profile of the surgeon scientist during the only world meeting exclusively focused on general thoracic surgery. During the 2014 Annual Meeting in Copenhagen, for the first time in Europe, the ESTS leadership will award scholarships to support traveling fellowships in hosting academic institutions to facilitate exchange of expertise and knowledge among European highly qualified thoracic surgical centers.

Molecular biology for thoracic surgeons

Substantial progress in understanding basic mechanisms of lung disease—still a substantial public health problem—may help to move forward clinical management of our patients. Molecular biology bears the opportunity to move forward in the direction of personalized medicine not only for thoracic malignancies.

Writing in Nature in 1961, Astbury described molecular biology as: “...not so much a technique as an approach, an approach from the viewpoint of the so-called basic sciences with the leading idea of searching below the large-scale manifestations of classical biology for the corresponding molecular plan. It is concerned particularly with the forms of biological molecules and [...] is predominantly three-dimensional and structural—which does not mean, however, that it is merely a refinement of morphology. It must at the same time inquire into genesis and function” (7).

So how does this translate into the thoracic surgeons practice and how is this applicable in the pathologies we treat? The following article will summarize the significance and give an update on molecular biology tools for thoracic malignancies.

Molecular biology of NSCLC

There are two main types of lung cancer divided by histological, clinical and endocrine characteristics, NSCLC (80-85% of lung cancer) and small cell lung cancer (SCLC) (15-20% of lung cancer) (8). Lung cancers arise from lung epithelial cells, which accumulate genetic and epigenetic alterations resulting from chronic exposure to carcinogens in tobacco smoke. Epigenetic changes are accounted by aberrant promoter methylation or histone modifications resulting in alteration of transcription of genes without altering genetic code. Genetic alterations can occur at
the chromosomal level (loss or gain of genomic material, translocations, and microsatellite instability), and at the nucleotide level (mutations). These abnormalities that occurred to proto-oncogenes or tumor suppressor genes can affect both function and/or expression levels of the proteins causing up-regulation of survival pathways and out-growth of affected lung epithelial cells. NSCLC can be histologically classified into three other main subtypes according to recommendation of the World health organization (WHO) and the International Association for the Study of Lung Cancer (IASLC) (9). Lung adenocarcinoma is the most common subtype of NSCLC, accounting for about 40% of NSCLC. Later in 2011, an effort has been made to further sub-classify adenocarcinoma with the integration of clinical, radiological surgical, and molecular issues in addition to tumor histology (by the IASLC, American Thoracic Society, and European Respiratory Society) (4,10). A total of 70% of the NSCLC cases are diagnosed with stage IV and patients received platinum based chemotherapeutic agents as the first line treatment (11). Nevertheless, the molecular change in NSCLC is heterogeneous and leads to a broad range of disease outcomes even within the same histological subtype. In the last decades, there has been a development in high-throughput and high-resolution technology for determining genetic and epigenetic alterations in cancers such as next-generation sequencing (NGS) and genomic hybridization array, gene expression microarrays, and proteomics. This technology has brought several new insights into the molecular biology of NSCLC and further sub-divides NSCLC based on molecular changes.

**Epigenetic aberrations**

Hypermethylation of tumor suppressor gene promoters causes silencing of gene transcription by inhibiting the binding of transcription factors to the promoter. The modification of histones such as methylation, acetylation, phosphorylation, ubiquitylation and ADP-ribosylation also takes part in the epigenetic regulation of genes by altering accessibility of chromatin. Gene promoter methylation profiling by genome wide methylation analysis has revealed that the methylation profile of several tumor suppressor genes such as CDKN2A and adenomatous polyposis coli (APC) served as prognostic and predictive markers (12). Methylation profile can also be identified in circulating DNA secreted in the liquid specimens such as blood, sputum, and bronchoalveolar lavage (BAL) (12). The investigation of DNA methylation in these specimens will potentially provide advantage in early and non-invasive detection of NSCLC. The methylation of gene promoters was operated by the enzyme DNA methyltransferase (DNMT) which could serve as a therapeutic target. AzadG or 5-azacytidine (AzaC), DNMT inhibitors, were approved by the US Food and Drug Administration (FDA) for the treatment of myelodysplastic syndrome (12). Histone de-acetylase inhibitor such as vorinostat has shown clinical activity in several cancers and is being tested in NSCLC under phase I/II clinical trials in combination with other agents (13).

**Genetic aberrations**

NSCLC is a cancer with high level of gene instability. Structural alterations of chromosomes (gain, loss, translocation) in the region containing important oncogenes and tumor suppressor genes causes gain or loss of the allele (14). Loss of heterozygosity (LOH) where one allele of a tumor suppressor gene such as CDKN2A and TP53 is missing has been involved in the initiation of malignant transformation of lung (15). Several oncogene mutations “driver mutations” have been discovered (16). Driver mutations are mutations that provide growth advantage to tumor cells and are critical for tumor cell survival. Tumor cells become dependent on driver mutations (addicted) therefore can serve as predictive factors and targets for therapy. Well established driver mutations include EGFR, KRAS, HER2, BRAF, PIK3CA, AKT1, MEK1, NRAS, echinoderm microtubule-associated protein-like 4 (EML4) ALK and MET amplification (16). As the results; novel targeted therapy of NSCLC has been intensively developed based on the driver mutations and their corresponding molecular pathways and has been shown to improve clinical outcomes significantly. The promising treatment of NSCLC patients with EGFR mutations with the EGFR tyrosine kinase inhibitor, gefitinib and erlotinib led to the recommendation to test all patients with advance adenocarcinoma for EGFR mutation [accounting 20% of NSCLC (8) for the selection of treatment (4)]. On the other hand, patients with KRAS mutations (10-30%) or amplification of proto oncogenes such as MET receptor tyrosine kinase (7-21%) of NSCLC patients confer the resistance to treatment with these tyrosine kinase inhibitors (8). Recently, the discovery of EML4-ALK fusion oncoprotein has shed light on the treatment of NSCLC with EML-ALK fusion [accounting for about 6.7% of NSCLC (17)] with ALK tyrosine kinase receptor inhibitor. All the patients harboring EML-ALK fusion responded rapidly to these inhibitors such as crizotinib (PF-02341066) (18) which was
later approved by the US FDA in 2011.

**micro RNAs (miRNAs)**

miRNAs are small RNAs which do not encode protein, however they play a key role in the regulation of gene expression. miRNAs bind to the 3’-untranslated region of mRNA of the target gene by using imperfect complementary sequence and induce the degradation of target miRNAs or block their translation into protein. After the first finding of the involvement of miRNAs in human diseases and cancer in 2002, almost 2,000 miRNAs with identified targets have been discovered to date. miRNAs are stable molecules and can also be analyzed in blood, liquid effusions as well as archived formalin fixed paraffin embedded tissues. Using high throughput methods such as miRNA profiling by PCR assay or miRNA arrays or sequencing, miRNA expression signature has been developed for several purposes such as diagnosis, prognosis, chemotherapeutic resistance, and response (19). miRNA Let-7, functions as tumor suppressor and the reduced expression correlates with poor prognosis of NSCLC. Let-7 targets to suppress the expression of several oncoproteins such as Myc proto-oncogene protein and Ras (19). miR-372 acts as oncogene has been shown to be involved in the neutralization of cyclin dependent kinases (CDK) inhibition by p53 (20). Later miRNA signature (hsa-let-7a, hsa-miR-221, hsa-miR-137, hsa-miR-372, and hsa-miR-182*) was discovered to be associated with survival and relapse of NSCLC patients (21). Antagonists to oncogenic miRNAs which overexpress or replacement of tumor suppressor miRNAs lost in cancer provide attractive treatment approaches. In vitro experiment and systemic delivery in in vivo pre-clinical models revealed efficient anti-tumor activity and was well tolerated (22). After several successes in pre-clinical models, a multicenter phase I study of miR-34 injected in liposome (MRX34) is being conducted in hematologic malignancies and solid tumors (ClinicalTrials.gov Identifier: NCT01829971).

So far it has been realized that targeting specific molecules or molecular pathways personalized to individual defects of each tumor provides the most effective treatment.

**Molecular biology of malignant pleural mesothelioma (MPM)**

MPM is one of the most aggressive tumors mainly derived from the pleura and predominantly associated with exposure to asbestos which remains the major causative factor. It is usually resistant to conventional therapies, and the prognosis of patients is particularly severe. Despite remarkable effort to determine the molecular mechanisms involved in MPM carcinogenesis, to date, findings have not led to develop an appropriate treatment approach and the molecular basis for this malignancy suffers from the relative lack of strong evidence.

**Intrinsic resistance and poor response to the traditional treatment modalities (i.e., surgery, radiation, and chemotherapy)** have driven researchers to explore thoroughly the molecular mechanisms that underlie mesothelioma carcinogenesis.

Over the past decade, emerging evidences have pinpointed derangements in several cellular pathways that involve cell cycle regulators, apoptosis, growth factors, and angiogenesis [reviewed in Zucali et al., 2011 (23)]. Epigenetic modifications also have been explored in MPM to dissect alterations of genes expression [reviewed in Vandermeers et al., 2013 (24)] even though promoter methylation coupled with gene expression modification is a frequent phenomenon in MPM, thus playing a role in oncogenesis, no valid biomarkers have been identified for diagnosis or prognosis.

Here we address new insights into molecular characteristics and the major genetic alterations that have been associated with MPM. Exploring this active area of research may have a great impact on the identification and the development of new therapeutic targets.

**Chromosomal alterations**

Karyotypic studies and comparative genomic hybridization (CGH) analyses using MPM specimens and cell lines have shed light on the genetic alterations involved in the carcinogenesis of MPM. Although there is no specific chromosomal aberration common to all cases of malignant mesothelioma, numerous prominent sites of chromosome loss have been described [reviewed in Carbone et al., 2002 (25)]. These include monosomy of chromosome 22 (26) which is the most frequent alteration found in 70% of MPMs [reviewed in Carbone et al., 2002 (25)]. Moreover, recurrent regions of loss have been identified at 1p, 3p, 6q, 9p, 13q, 14q, 15q, 17p, and 22q (27,28). This pattern of chromosomal loss emphasized the possible involvement of suppressor genes (TSG) in the initiation and the progression of MPM malignancy.
Gene mutations

New methods, known as NGS, have revolutionized the genetic analysis of mesothelioma (29). Transcriptome analysis was used to characterize particular genes, mutational profile for four to MPM tumors that do not exist in control tissue. Using this approach, the authors identified uncharacterized human cancer mutations including somatic mutation, gene deletions or silencing, and RNA editing (30). Indeed, deeper investigations in sequencing should provide precious guidance to detect true-driver mutations in MPM to target for efficacious therapy. Only a couple of gene mutations critically located on chromosomes 22q, 9p, and 3p have shown a high rate in MPM malignancy.

The two most abundant alterations in MPM concern CDKN2A and NF2. Indeed, mesothelioma lack expression of both CDKN2A encoded proteins, p16 and ARF, due to gene deletion or methylation [reviewed in Jean et al. (31)].

Mutations in the tumor suppressor neurofibromatosis type II (NF2; Merlin) gene have been found in 40% of mesothelioma (32-34). In MPM tumors with no detectable genetic alterations of NF2, its activity is downregulated. It was observed that expression levels of the protein kinase C-potentiated phosphatase inhibitor of 17 KDa (CPI-17), which inhibits the phosphatase reactivating NF2, were significantly higher in 17% of MPM with no detectable NF2 mutations (35). Furthermore PTEN immunostaining was shown to be absent in over 60% of MPM patients and correlated with worst outcome (36). Absence of PTEN would result in increased AKT activity and NF2 degradation. It has been proposed that genes which are inactivated in a given tumor type and directly regulate tumor growth by either inhibiting growth or promoting death are “gatekeeper” genes (37). According to the data mentioned above, NF2 does correspond to this definition and should be considered a “gatekeeper” in mesothelioma. A role of NF2 in tissue repair is supported by the observation that the active form of NF2 suppresses tumorigenesis by migrating into the nucleus where it inhibits the E3 ubiquitin ligase CRL4 and through that controls a subset of Hippo pathway target genes (38). This observation is consistent with previous evidence of NF2 signalling-dependent activation of the Hippo pathway (39). The latter is an essential regulator of cell proliferation during tumorigenesis (40) [reviewed in Book chapter Mesothelioma by Felley-Bosco and Opitz in (41)].

Cyclin-dependent kinase inhibitor 2A (CDKN2A) is a tumor suppressor gene that maps to the 9p21 chromosomal locus that encodes p16, a CDK inhibitor regulating cell cycle during G1/S phase as well as other alternate transcript variants (42). CDKN2A is homozygously deleted at high frequency in MPM cell lines and tumor specimens (33,43-46). Cells lacking p16 lose their cell cycle control and undergo a malignant transformation. Indeed, increasing evidence suggests that CDKN2A loss could be used as negative prognostic factor, and represents an ideal candidate for gene therapy (41).

In the last decade, investigations have been conducted to identify BAP1 gene (BRCA1 associated protein-1) as a tumor suppressor gene that is frequently lost in MPM (47,48). BAP1 maps to the 3p21.1 locus and encodes for an ubiquitin COOH-terminal hydrolase. It is believed that BAP1 mediate its effects through chromatin modulation, transcriptional regulation, and possibly via the ubiquitin-proteasome system and the DNA damage response pathway (49). Growing evidence from clinical and molecular studies suggested that when individuals with BAP1 mutations are exposed to asbestos, mesothelioma predominates. Those authors pointed the fact that BAP1 mutation, alone, might be sufficient to cause mesothelioma (47,50).

The TP53 gene controls cell cycle and apoptosis and is mutated in many types of human cancers, and in about 20% in human MPM, a fairly low rate in comparison with other human cancers (51). Point mutations are the main types of alterations in mesothelioma. The International Agency for Research on Cancer p53 database indicates 6 point mutations, 5 missense mutations, and 1 stop mutation (http://www.p53.iarc.fr, accessed June 11, 2011) (31). The clinical significance of TP53 mutations remains unclear so far.

Acknowledgements

We thank Mayura Meerang, Karima Bérard, and Chloé Spichiger for supporting us during edition and revision of the article.

Disclosure: The authors declare no conflict of interest.

References


32. Sekido Y, Pass HI, Bader S, et al. Neurofibromatosis type 2 (NF2) gene is somatically mutated in mesothelioma but

Cite this article as: Opitz I, D’Amico TA, Rocco G. The biomolecular era for thoracic surgeons: the example of the ESTS Biology Club. J Thorac Dis 2014;6(S2):S265-S271. doi: 10.3978/j.issn.2072-1439.2014.05.01