Symposium article

Adenocarcinoma, a molecular perspective

R. A. Stahel*

Clinic and Policlinic of Oncology, University Hospital, Zürich, Switzerland

introduction

Adenocarcinoma of the lung is currently the major histological subtype in East Asia and the USA and is surpassing the frequency of squamous cell carcinoma in some European countries. Although in most instances it occurs in smokers or former smokers, it develops more frequently than any other histological subtype in patients who have never smoked. Based on recent investigations into the nature of adenocarinoma of the lung, it has become obvious that several distinct molecular diseases are lumped together under this morphological entity. This article reviews the molecular characteristics that may influence clinical decision making in the future.

ras

The first molecular changes found to be associated with adenocarcioma of the lung were mutations of the Kras oncogene. The ras proteins are members of a large superfamily of guanine tri-phosphate (GTP)-binding proteins involved in signal transduction regulating cell growth. Knockout studies in mice have demonstrated that Kras, but not Hras or Nras, is required for normal mouse development [1]. The conditional expression of mutated Kras in mice models resulted in the formation of lung adenomas, thus confirming its importance in lung tumorigenesis [2, 3]. Kras mutations have been identified in 30% of lung adenocarcinoma [4]. Mutations of Kras codon 12 with G to T transversion were exclusively seen in patients with tobacco-associated adenocarcinoma of the lung [5].

Patients with Kras-mutated tumours have a worse prognosis after resection than patients with wild-type tumours [6, 7]. The negative prognostic impact of ras mutations has been demonstrated in several studies and confirmed in a recent meta-analysis [8]. Mutations of ras may be predictive of resistance to chemotherapy. The adjuvant trial JBR.10 stratified patients according to the presence of ras mutations. In this trial adjuvant chemotherapy did not seem to confer a survival benefit in ras-mutated tumours; however, this was not significant in the interaction analysis [9].

The stable localization of ras proteins to the plasma membrane by the covalent attachment of a farnesyl isophenoid group is an essential first step for the biologic activity of ras. Thus inhibition of this farnesylation was an obvious target for therapies directed to tumours with ras mutations. However, clinical studies with farnesyltransferase inhibitors in lung cancer gave disappointing results, most likely due to fact that Kras can be modified by alternative enzymes.

Micro-RNAs are small non-coding RNAs that repress their target RNAs by complimentary base pairing. Ras is regulated by the *let-7* microRNA family. In lung tumours let-7 expression is lower than in normal lung tissue, while ras protein expression is significantly higher, providing a potential role for *let-7* in lung cancer [10]. Low expression of let-7 RNAs was found to be associated with poorer survival in lung adenocarcinoma [11]. However, these data on the potential role of microRNAs in lung adenocarcinoma are preliminary and need confirmation.

epidermal growth factor receptor

For clinicians, interest in the molecular characterization of lung adenocarcinoma has increased due to the finding that mutations of the tyrosine kinase binding domain of the epidermal growth factor receptor (EGFR) are associated with dramatic responses and clinical benefits with the EGFR tyrosine kinase inhibitors gefitinib and erlotinib. EGFR is one of the four types of the family of ErbB receptors, together with HER2, HER 3 and HER4. Up to now, 11 ligands of this receptor have been identified, including epidermal growth factor (EGF) and transforming growth factor alpha (TGFα) [12]. Mutations of EGFR can be classified according to sensitivity to the presently known EGFR tyrosine kinase inhibitors. The majority include in-frame deletions of exon 19. The second most frequent are point mutations, mostly L858R in exon 21, more seldomly G719A/C in exon18, which are also associated with response to EGFR tyrosine kinase inhibitors [13, 14]. Up until now, three kinase domain mutations were found to be associated with resistance to EGFR tyrosine kinase inhibitors, the most common being the exon 20 point mutation T790M.

EGFR mutations are oncogenic *in vitro* and *in vivo*. This was proven by transforming lung epithelial cells with mutated EGFR [15] and by the use of transgenic mice models where introduction of mutated EGFR into the lung epithelium did lead to the formation of adenocarcinomas [16, 17].

Collective data from many investigators have demonstrated the association of mutations with lung adenocarcinoma, absence of smoking history, East Asian ethnicity and female gender [13, 14]. Investigations of EGFR mutations in tumour samples from 617 patients mostly from East Asia, and a minority from Australia and the USA, identified EGFR mutations in 21%, virtually all in adenocarcinomas. Mutations were more frequently in never smokers (51 versus 10%), East Asian ethnicity (30 versus 8%) and females (42 versus 14%) [13, 18]. Kras mutations were detected in 8%, but not in any

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tumour with EGFR mutations. This and other investigations suggested that two distinct molecular pathways lead to lung adenocarcinoma, tobacco exposure resulting in Kras-mutated tumours and exposure to another unknown carcinogen to EGFR-mutated tumours [19–21].

Available data on the prognostic value of EGFR mutations are conflicting. While the molecular analysis of patients treated in the TRIBUTE and INTACT trials suggested a prolonged survival in patients with mutations [22, 23], no significant differences were found in patients undergoing resections of non-small cell lung cancer [18] or lung adenocarcinomas [24]. In contrast, there is no doubt about the predictive value of EGFR mutations in tumour response, which ranged between 65–92% in patients with mutations and 9–13% in patients without mutations [25].

HER2

Binding of ligands to the EGFR receptor results in the formation of homo- and heterodimers, including HER2, which has no ligand-binding capacity, as partner. In cell lines overexpression of HER2 as been shown to confer sensitivity to EGFR tyrosine kinase inhibitors [26] and results of clinical investigations point in a similar direction [27]. Mutation of HER2 has been identified in 4% of 120 primary lung tumours and, among adenocarcinomas, the frequency was 10% [28]. Subsequent investigations demonstrated that in lung adenocarcinoma HER2 mutations, EGFR and Kras mutations were mutually exclusive [29]. *In vitro* studies also documented that cells carrying HER mutations remain sensitive to HER2 inhibitors such as lapatinib but become resistant EGFR inhibitors [30].

BRAF

RAF is a serine-threonine-specific protein kinase that is activated downstream of the ras protein. It activates the MAP kinase pathway. Somatic activating mutations of BRAF were identified in human cancers, in particular in melanoma where it occurs in up 70%. Missense mutation in the kinase domain has also been identified 11% of 35 in lung adenocarcinoma cell lines [31] and in 2 of 127 lung adenocarcinoma tissues [32]. From the available data it appears that mutations of BRAF, EGFR and Kras are also mutually exclusive.

expression profiling

Unsupervised hierarchical clustering in expression profiling allows in molecular classification of tumours based on the similarity of gene expression. Several groups of investigators have examined lung carcinomas using this methodology. A common feature of all studies was that the gene expression profile recapitulated the known histological subtypes [33] and that lung adenocarcinomas fell into distinct subclasses [24, 34–37]. These studies suggest the development of lung adenocarcinoma to occur in at least two different pathways, with one including Clara cell or terminal respiratory unit differentiation, the other being of a more poorly differentiated

nature. EGFR mutations were found to be more common in tumours with the terminal respiratory unit differentiation whereas Kras mutations were more common in tumours of the poorly differentiated subgroup [24].

Using supervised analysis, several independent studies have identified gene expression profiles associated with outcome after surgery. These include studies including all histologies of non-small cell lung cancer [38, 39], as well as studies restricted to lung adenocarcinoma [37, 40]. A recent meta-analysis of gene expression signatures from several datasets identified 64 genes whose expression was significantly related with survival after surgery for stage I non-small cell lung cancer [41].

conclusion

Adenocarcinoma of the lung comprises several molecular entities that are currently being defined. Available evidence suggests a least two major pathways of development to lung adenocarcinoma; one associated with Kras mutation, another with EGFR or to a lesser extent HER2 or BRAF mutation.

Mutations of Kras are associated a smoking history. They are mutually exclusive with EGFR mutations, Her2 mutations and BRAF mutations, which have been identified in adencarcinomas of non-smokers.

With the availability of EGFR tyrosine kinase inhibitors in the clinic, the need for the identification of the molecular characteristics of the tumour of an individual patient is becoming increasingly accepted by investigators in the field and might become standard in the near future. The molecular characteristics of adenocarcinoma will need to be taken into account for the development of additional therapeutic approaches. Gene expression arrays are likely to become an integrated part of clinical decision making.

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