Review paper

Genetic biomarkers in lung cancer therapy – an update

JOANNA CHOROSTOWSKA-WYNIMKO, ADAM SZPECHCINSKI

Laboratory of Molecular Diagnostics and Immunology, National Institute of Tuberculosis and Lung Diseases, Warsaw, Poland

Abstract

Biomarkers are objectively measured indicators of pathogenic processes of disease development or responses to a therapeutic intervention. It is commonly believed that development of reliable biomarkers for lung cancer both prognostic and predictive would considerably change disease diagnostics and treatment outcomes. Paper reviews current data on recent development in clinical applicability of molecular biomarkers in lung cancer.

Key words: *biomarker, lung cancer, EGFR.*

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From lung carcinogenesis to genetic biomarkers

Technical progress substantially increased our knowledge and understanding of the key role that particular genes play in the pathogenesis of lung cancer. Numerous studies confirmed that the modified expression of genes regulating main biological processes like cell cycle, differentiation, maturation, aging and apoptosis is of decisive significance [1]. It is acknowledged now that unrestrained growth of tumor tissue results directly from the increased activity of oncogenes as well as down-regulated expression of the tumor suppressor genes (TSGs) due to the genetic (mutations) or epigenetic (hyperexpression, methylation) modifications [2]. Since it is possible to effectively detect these alterations in human tissues, some of them might serve as reliable diagnostic markers for cancer screening.

The main mechanisms regulating growth and invasiveness of tumor are reflected in the enhanced or changed expression of particular genes. For example, modified activity of the ERBB gene family encoding EGFR (epidermal growth factor receptor) and HER2/neu (human epidermal growth factor receptor 2) is responsible for the diminished requirement of NSCLC for the growth factors [1, 2]. Similarly, apoptosis that serves as a physiological mechanism regulating cells liveliness, especially in those with disrupted or abnormal DNA structure, was shown to be inhibited as a result of deregulated expression of p53 and bcl2 genes (respectively in 50% and 30% of NSCLC and more than 90% of SCLC). Other typical alterations of gene expression result in resistance to paracrine growth regulation (loss of heterozygosity (LOH) *TP53*, *p16*), increased angiogenic activity of cancer tissue (VEGF genes), up-regulated tumor cells replication (telomerase gene) as well as augmented ability to invade neighboring tissue and metastasize (laminin and integrin gene) [3].

The number and type of modifications in gene expression parallels cancer development [4]. In addition, certain molecular markers seem characteristic for particular phases of tumor growth and metastasis formation, defining the transition from mild to moderate and severe atypia and subsequently to *carcinoma in situ* (CIS) and microinvasive carcinoma [5].

Early modifications (3p LOH, 9p21 LOH) might be detected as soon as minor lesions such as hyperplasia or dysplasia occur in the bronchial mucosa [6]. Some of them, mostly promoters methylation, have been observed also in normal mucosa of chronic smokers. More significant changes in biomarker expression are found in preneoplastic lesions, in dysplasia or *carcinoma in situ*. Late modifications, typical for invasive cancer, are more abundant and diverse from loss of genetic material (alleles), spontaneous or induced mutations to epigenetic modifications like genes hyperexpression or

Correspondence: Joanna Chorostowska-Wynimko, Laboratory of Molecular Diagnostics and Immunology, National Institute of Tuberculosis and Lung Diseases, 26 Plocka Str, 01-138 Warsaw, Poland. Phone number: +48 22 431 21 58, Email: j.chorostowska@igichp.edu.pl

methylation. Smoking is particularly effective in inducing multiple genetic modifications in the airways. Active carcinogens present in the cigarette smoke directly interact with the k-ras, p53 and FHIT genes critical for the tumor development and induce the earliest carcinogenic modifications – DNA hipermethylation and deletions in the TSGs [7]. Chronic exposition to the cigarette smoke is also responsible for the accumulation of these modifications, increasing therefore the probability of preneoplastic or neoplastic lesions occurrence in the bronchial mucosa. Thus, smoking cessation is rightly regarded as one of the most important methods of lung cancer prevention.

Due to these sequential and progressively expanding changes during the lung cancer development, the expectances toward genetic biomarkers as the promising diagnostic tool are increasing. Molecular biology techniques might effectively estimate expression of particular, appointed genes not only in tumor cells, but also in other materials like sputum, bronchoalveolar lavage (BAL) and serum/plasma. Number of studies evaluating diagnostic efficiency of different markers or more often marker panels has tremendously increased over the last decade. However, at present there are no widely accepted biomarkers of reliable and confirmed diagnostic value [8, 9].

Clinical implications of genetic markers

Apart from lung cancer screening and diagnostics, molecular markers transpire as a new hope for improved disease prognosis in patients beginning or currently undergoing chemotherapy [10].

Several groups attempted to evaluate a prognostic role of biomarkers in overall survival. However, available data are contradictory and inconclusive. Ramirez et al. observed that K-*ras* mutation in the serum of 12 out of 50 resected NSCLC patients significantly correlated with survival [11]. Also, Kimura et al. found a considerable association between the presence of mutant K-*ras* in plasma and objective responses relevant for the overall survival [12]. Inconsistently, Camps et al. showed no correlation between the presence of mutant K-*ras* genotype in serum and objective response rate, progression-free survival, or overall survival [13]. Moreover, tendency towards the better response rate and survival in patients with circulating mutant K-*ras* was observed. Similarly, it has been demonstrated that structural mutations of *TP53* in the tumor cells, *APC* promoter methylation as well as down-regulated expression of HIN-1 gene strongly correlated with poor survival of lung cancer patient [14]. Also plasma DNA concentration has been reported to significantly correlate with elevated serum lactate dehydrogenase levels, advanced tumor stage and poor survival in the group of 185 NSCLC [10]. However, practical prognostic impact of above-mentioned biomarkers still remains unclear and needs further evaluation.

Similarly, a reliable serologic biomarker that might be supportive in predicting the treatment response of NSCLC patients is not available yet. Several molecular diagnostic methods have been evaluated for their applicability in the assessment of lung tumor susceptibility to chemotherapy. Presence of cysplatine adducts in the cytoplasm of normal cells, as well as the decreased expression of ERCC1 or Ape1 genes seem to be a reliable and relatively easy method to estimate cancer cells resistance to cytostatic drugs. Likewise, Rosell et al. have proven that high expression of the RRM1 (ribonucleotide reductase responsible for the DNA synthesis and repair) gene closely corresponded with better outcome of surgical treatment, lower rate of subsequent tumor relapse and much prolonged patients survival time [15]. Another study showed that SNP (single nucleotide polymorphism) in the plasma MTHFR (methylenetetrahydrofolate reductase responsible for DNA methylation) is associated with slight differences in median time-to-progression (TTP) in cisplatin/gemcitabine-treated patients with NSCLC [16]. Patients with the MTHFR 677CC genotype presented almost two month longer TTP than those with CT and TT genotypes. Ramirez et al. have demonstrated that methylation of 14-3-3σ in serum might be a valuable prognostic factor for survival in NSCLC patients receiving platinum-based chemotherapy [17]. The 14-3-3σ methylation-positive patients (39 out of 115) showed significantly better median survival than patients with sera negative for 14-3-3σ methylation (15.1 months vs. 9.8 months, respectively). Moreover, the risk of death for 14-3-3σ methylation-negative responders was almost five times that of 14-3-3σ methylation-positive responders. Also, circulating nucleosomal DNA in combination with oncological biomarkers: carcinoembryonic antigen (CEA) and CYFRA21-1 proved its potential value as an early predictor of chemotherapy efficacy [18].

The only molecular test currently validated for the implementation into the clinical practice is the mutational analysis of EGFR gene [19]. Epidermal growth factor receptor (EGFR) is the molecule of high interest with regard to EGFR-targeted treatment with tyrosine kinase inhibitors (TKI) and its certain mutations are established as a marker of patient's response to chemotherapy. Accordingly, Clarke et al. were one of the first to report elevated EGFR mRNA in the peripheral blood of 30% NSCLC patients [20]. Next, Kimura et al. detected two major somatic EGFR mutations in serum DNA from 13 out of 27 (48.1%) NSCLC patients [21]. EGFR mutation positive patients presented better outcomes with gefitinib treatment than EGFR mutation negative group. Other authors consistently confirmed that in adenocarcinoma clinically meaningful mutations comprise of base-pair deletion at exon19 (del746_A750) and a point mutation at exon 21 (L858R) [22, 23]. Both result in ligand-independent tumor cell dependence on EGFR signaling, therefore enabling EGFR-TKI effectiveness. It was shown that these mutations are more frequent in women, of Asiatic origin, non-smokers, diagnosed with adenocarcinoma [24]. Following clinical studies confirmed increased responsiveness, overall survival and tumor free-survival in EGFR

mutation positive lung cancer patients. Initially, EGFR TKIs efficacy has been demonstrated in the second-or third line therapy of lung cancer in patients with confirmed EGFR mutations or increased EGFR copy numbers [25, 26]. Recently, Mok et al confirmed that first-line therapy in mutation positive advanced adenocarcinoma patients resulted in better response rate (35.5% vs. 24.4%) and significantly longer progression free survival (29.4 weeks vs 23.4 weeks, $p < 0.0002$) in erlotinib vs. placebo treated group [27]. Similarly, gefitinib assigned as a first-line therapy in treatment naive, EGFR mutation positive patients with advanced adenocarcinoma allowed significantly prolonged progression-free survival in comparison to standard carboplatin-paclitaxel therapy group [28]. It should be mentioned however, that not all EGFR mutations have similar biological effect. Some, as mutations in exon 20, are related to intrinsic resistance of tumor cells. Moreover, activating mutations of KRAS gene located downstream might cause similar effect of intrinsic resistance to EGFR TKIs. There is also a phenomenon of secondary resistance due to the T790 M specific mutation in EGRF gene or amplification/overexpression of MET gene. Secondary resistance is responsible for TKI-resistant relapse following previously successful treatment with TKIs. Taking above into account it is rather obvious that screening for activating EGFR mRNA or rather DNA mutations predetermining results of treatment with EGFR tyrosine kinase inhibitors will achieve considerable clinical relevance in the near future.

As for the surgical treatment of NSCLC, it is expected that analysis of circulating DNA might prove useful for the post-operative follow-up of NSCLC patients [29]. Monitoring of free plasma DNA concentration has been shown to provide quite valuable information concerning disease recurrence or effectiveness of radical treatment NSCLC. Sozzi et al. confirmed that successful radical tumor resection resulted in significantly lower concentration of plasma DNA than in non-surgically treated patients (7.1 vs. 24.7 ng/ml) [30]. Also, its quantification and molecular characterization was shown to correlate closely with the early recurrence events during the follow-up. In relapse-free individuals circulating DNA concentration was significantly lower than before surgery, while in patients with lung cancer recurrence or metastases up to 20-fold increase was observed together with microsatelite alterations (loci 3p14.2, 3p21, 3p23, 3p24.2, 3p25-26) persistent throughout follow-up period.

Significant correlation between *p16* methylation rate, survival and disease-free survival at 12-month postoperative follow-up has also been reported [31]. The NSCLC patients with *p16* methylation demonstrated in plasma and pre-resection pleural lavage (14.3% and 21.4% of 14, respectively) had shorter survival.

Summary

While discussing the clinical implications of the extensive search for the molecular biomarkers useful in lung cancer, it should not be forgotten that it is also closely related to the investigation on the new treatment modalities. Many known biomarkers represent key mechanisms required for consecutive stages of tumor development, such as modified requirement for the growth factors or resistance to cell growth and apoptosis regulation. Better understanding of these mechanisms due to the intensive search for the reliable early stage biomarkers might significantly help in elaboration of new treatment concepts.

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