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Biomarkers in lung cancer

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1. ABSTRACT

Here we review the role of tissutal and circulating biomarkers in the management of lung cancer. In the past they were considerate quite ineffective tools as regards prognosis and prediction of treatment activity, nowadays instead, they are becoming a crucial key point as potential predictive issues in driving therapy, with possibly prognostic values as well.

2. INTRODUCTION

Lung cancer is the major cause of cancer-related deaths in the world (1). Two main pathological entities of lung cancer are recognized depending on cell type: small cell (SCLC) and non-small cell lung cancer (NSCLC), overall 25% and 75%, respectively (2). Lung cancer is generally diagnosed at advanced stage, and is associated with poor prognosis, with a median survival of 8-10 months (3). If for SCLC few improvement has been achieved in the last decade, for NSCLC overall survival has been significantly prolonged due to the discovery of new therapeutic agents. NSCLC is further divided in three major histological subtypes: squamous cell carcinoma, adenocarcinoma and large cell carcinoma (4).

Adenocarcinoma is a malignant epithelial tumor with glandular differentiation or mucin production, showing acinar, papillary, bronchoalveolar, or solid with mucin growth patterns or a mixture of these patterns. Common molecular characteristics of NSCLC adenocarcinoma are a higher thymidylate synthase expression than in squamous cell carcinoma (5), frequent point mutations of dominant oncogenes, such as k-RAS genes and p53 and p16Ink4, higher expression of TTF-1 (up to 75% of adenocarcinomas) (4).

Squamous cell carcinoma is a malignant epithelial tumor showing keratinization and/or intercellular bridges that arise from bronchial epithelium. These features vary with degree of differentiation, being prominent in well-differentiated tumors and focal in poorly differentiated tumors and focal in poorly differentiated tumors. Usually k-RAS mutations are rare in squamous cell carcinoma, as well as HER2/Neu expression; most squamous cell carcinomas demonstrate large 3p segments of allelic loss, whereas most adenocarcinomas have smaller chromosome areas of 3p allele loss (4)

Large cell carcinoma is an undifferentiated NSCLC that lacks the cytologic and architectural features

of small cell carcinoma and glandular or squamous differentiation. K-RAS mutations, p-53 mutations and Rb pathway alterations occur with the same frequency as in other NSCLC subtypes (4).

Tumor markers have been extensively studied in lung cancer, but none is specific for this malignancy. Neuron-specific enolase (NSE) is the tumor marker of choice in SCLC (6), whereas there is no specific tumor marker for NSCLC; nevertheless most of the circulating markers in patients affected by lung cancer have no prognostic or predictive value. On the contrary, tissutal markers may give a hint about tumor genotype that together with host characteristics could influence the treatment algorithm. Summarizing, we divided markers for SCLC and NSCLC, and circulating markers and tissue markers.

3. CIRCULATING MARKERS

The role of most of serum markers for lung cancer remains undefined. Several markers, such as carcinoembryonic antigen (CEA), small cell carcinoma, as well as cytokeratins including CYFRA 21-1, tissue polypeptide antigen (TPA), and Ca 15-3 have been proposed as useful contributions to the diagnosis of lung cancer, although their role has not been demonstrated clearly (7). NSE is specifically used in SCLC only (8).

Up to now, the most accepted clinical use of tumor circulating markers in lung cancer is in the follow-up, with contradictory results in relation to their possible prognostic or predictive value.

3.1. Small cell lung cancer

SCLC usually arises as a rapidly growing tumor of the major airways and it is considered the anaplastic variety of this spectrum of neuroendocrine (NE) tumors. TTF-1 is positive in the majority of both large and small cell NE carcinomas and is therefore useful to differentiate those lesions from poorly differentiated NE carcinomas of other sites. High molecular weight cytokeratins are not expressed in SCLC, in contrast with non-NE carcinomas (9).

3.1.1. Neuron-specific enolase

SCLC yields neuroendocrine properties that are considered to be part of its aggressive clinical behavior (10). However, the serum NSE level is not absolutely specific to the NE differentiation. Clinical experience has shown low levels of NSE in limited SCLC and high levels in NSCLC, mostly large cell, therefore there is concern about the sensitivity of this marker (11). Its role is for follow-up of the disease during treatment.

3.1.2. Chromogranin A

Chromogranin A (CgA) is a 49 KD acidic soluble protein, initially recognized in the core of the adrenal medullary cathecolamine storage vesicles (12). This protein is ubiquitously present in neuroendocrine cells and often found in the serum of patients affected by SCLC (13). Its role is in the follow-up of the disease during treatment and for prediction of recurrence.

3.2. Non small cell lung cancer

3.2.1. Cyfra 21-1

Cyfra 21-1 is a fragment of cytokeratin subunit 19 and can be measured in serum by an immuno-radiometric assay. The prognostic information provided by serum Cyfra 21-1 seems to be higher than the one produced by the cytokeratin 18 marker, or tissue polypeptide specific antigen (TPS) (14).

Other authors have demonstrated that CEA and Cyfra 21-1 are the most sensitive tumor markers in NSCLC (15-17). According to several authors the serum Cyfra 21-1 distribution is highest in squamous cell carcinoma, metastatic stage and poor performance status (18); however not all the authors agree with correlation between histology and serum levels of Cyfra 21-1 (19).

3.2.2. Ca 15-3

Episialin or MUC-1 is a transmembrane glycoprotein expressed at the apical side of normal glandular cells (20). It is expressed by most “wet” epithelia such as bladder, breast, stomach, pancreas, ovary and respiratory tract. In a tumor, the cell polarization is lost and the normal tissue architecture is disrupted by the growing neoplastic tissue, allowing MUC-1 to be shed into the circulation where it can be measured by immunoassays (21). MUC-1 mucin as detected by a Ca 15-3 sandwich capture assay had been the first marker to correlate with treatment response in breast cancer (22-25). As a matter of fact, Ca 15-3 is elevated in 54-80% of breast cancer patients, but it may rise even in other malignancies, e.g., lung, ovarian, endometrial, bladder and gastrointestinal carcinomas. *In vitro*, MUC-1 inhibits the E-cadherin mediated cell-cell adhesion system to extra cellular matrix (ECM), which stimulates the metastatic process (26). It has been suggested that MUC-1 and epidermal growth factor receptor (EGFR) expression are likely to be activated within different pathogenic pathways. In lung cancer, MUC-1 is highly correlated with adenocarcinoma, poor prognosis and tumor spreading (27, 28).

So far, the incidence and role of pathological Ca 15-3 serum levels in patients with lung cancer, especially adenocarcinoma, are still unknown.

We already suggested the possible correlation between low levels of Ca 15-3 and response to EGFR tyrosine kinase inhibitors (TKI) in patients affected by adenocarcinoma of the lung with bronchioloalveolar carcinoma features (29), nevertheless the possibly predictive role of Ca 15-3 needs further confirmation.

3.2.3. Carcinoembryonic Antigen

CEA is mainly found in adenocarcinomas, but it is unable to distinguish between NSCLC and SCLC (15, 16).
It has been proposed that, in case of SCLC with normal serum NSE levels, CEA and squamous cell carcinoma (SCC) should be normal as well (19). It is unclear if CEA could have a prognostic value, in particular for recurrence risk (30, 31).

3.2.4. Ca 19.9

The sensitivity of the mucins and among them, Ca 19.9., is lower than that of other tumor markers in NSCLC, however their highest concentration is found in adenocarcinomas. Some authors have reported the prognostic utility of Ca 19.9. in this disease, but those observations need confirmation (32)

3.2.5. Squamous cell carcinoma

SCC is a tumor marker with low specificity for lung cancer, but high relationship with NSCLC, mainly squamous cell histotype. It is used for monitoring NSCLC, although some reports discourage its routine use because of low sensitivity (33).

4. TISSUTAL MARKERS

Tissutal biomarkers are important for their potential use in customizing a therapy for NSCLC patients together other host factors.

The host factors include age, race, weight loss, performance status, gender, comorbidities, social factors; they all together concur to a personalized-driven therapy. The tumor characteristics are potentially as important as host factors; in particular genes and proteins with activity in peculiar biochemical pathways may be important in the identification of different risks for survival and other clinical relevant outcomes.

Up to now, their potential role involves predominantly NSCLC; for SCLC no putative biomarker has been recognized with predictive or prognostic value.

4.1. Excision repair cross-complementing-1

The nucleotide excision repair cross-complementing-1 (ERCC1) gene plays a key role in DNA repair after cisplatin damage. It is responsible for the 5' incision required for the removal of DNA adducts that are the basis for platinum cytoxicity (34). The balance of DNA damage to DNA repair dictates tumor cell death or survival after cisplatin therapy. ERCC1 mRNA levels are prognostic after surgical resection of NSCLC (35). ERCC1 mRNA levels are predictive of improved response and survival from cisplatin-based therapy (36).

Immunoistochemical positivity for ERCC1 is predictive of failure for adjuvant therapy platin-based in NSCLC according to analysis of 761 tissue samples of patients recruited in the International Adjuvant Lung Cancer trial (37).
It is still controversial if analysis of ERCC1 should be through immunoistochemistry or mRNA levels, or whether both the systems are equally significative and reliable.

4.2. RRM1

RRM1 is the molecular target of gemcitabine and a component of ribonucleotide reductase, which is required for deoxynucleotide production (38). RRM1 protein levels in tumor specimens are predictive of disease response in patients with advanced NSCLC and treated with gemcitabine (39).

4.3. BRAC1

A growing body of evidence suggests that the BRAC1 confers sensitivity to apoptosis induced by antimicrotubule drugs (paclitaxel and vincristine) but induces resistance to DNA-damaging agents (cisplatin and etoposide) (40-42). Gene expression signatures have been reported to predict survival outcome in resected stage I (43). In particular, Rosell *et al* reported that BRCA1 mRNA expression is closely related to ERCC1 and RRM1, but it is the most significant prognostic marker of relapse in patients affected by NSCLC stage I and resected (44).

4.4. Epidermal growth factor receptor

The EGFR family of genes encodes transmembrane molecules that have been implicated in the development and progression of cancer (45, 46). After ligand binding, the transmembrane receptor forms homodimers or heterodimers, internalizes, and autophosphorylates tyrosine residues in its cytoplasmic domain, thereby triggering a cascade that leads to cellular proliferation, angiogenesis, metastasis, and inhibition of apoptosis. The EGFR gene is frequently expressed in solid tumors, and NSCLC as well- Non–small-cell lung cancer frequently expresses EGFR (47). At present, somatic activating mutations in EGFR tyrosyn kinase (TK) domains (exons 18-21) represent the major molecular determinant of the clinical response to treatment with TKI, Gefitinib or Erlotinib (48, 49). Increasing clinical evidence suggests that patients with EGFR mutations may receive more benefit from EGFR TKI therapies than chemotherapy (50, 51); on the other hand, KRAS mutations, which have been found to be mutually exclusive from EGFR ones, are associated with lack of response (52). Recently, mutations in the kinase domain of ERBB2 have been detected in a small fraction (2-4%) of NSCLC (53). The presence of such mutation has been implicated in TKI sensitivity (53, 54). NSCLC tumors that over express both EGFR and HER2 are more sensitive to EGFR TKI than are tumors that over express EGFR but are HER2 negative (55).

4.5. k-RAS

Somatic mutations of the k-RAS oncogene have been assessed as a mechanism of de-novo resistance to EGFR TKI in patients with NSCLC, and to anti-EGFR monoclonal antibodies in patients with metastatic colorectal cancer (mCRC). A systematically review of 252 manuscripts about expression of k-RAS in NSCLC showed that k-RAS mutations are highly specific negative predictors of response to TKIs in advanced NSCLC; and similarly to anti-EGFR monoclonal antibodies alone or in combination with chemotherapy in patients with mCRC (52).

The authors in the meta analysis suggested that the low sensitivity of k-RAS mutations for determining non-responsiveness to TKI clearly shows that additional mechanisms of resistance to EGFR inhibitors exist.

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Abbreviations: SCLC: small cell lung cancer, NSCLC: non small cell lung cancer, NSE: neuron specific enolase, CEA: carcinoembryonic antigen, TPA: tissue polypeptide antigen, NE: neuroendocrine, CgA: Chromogranin A, TPS: tissue polypeptide specific, ECM: extra cellular matrix, EGFR: epidermal growth factor receptor, TKI: tyrosine kinase inhibitor, SCC: squamous cell carcinoma, ERCC1: excision repair cross-complementing-1, BRAC1: breast cancer susceptibility gene 1, TK: tyrosine kinase, m-CRC: metastatic colorectal cancer.

Key Words: Markers, Lung, Cancer, Tumor, Review

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