

# Research Article

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## Biomarkers of Lung Cancer: Meta-analysis of Biomarkers Used to Identify Types of Lung Cancers Based on the Morphology and Histology

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### ABSTRACT

Cancer of the lung is a malignant lung tumor with a wide range of histological variants. The tumor arises from different types of cells, such as bronchioles, epithelium, bronchial mucous glands, or the alveoli. How effective the treatment is depends on the histological variant of the lung tumor. It is therefore necessary that the histology of the cancer and the respective biomarkers be accurately identified. Detection of malignant cells is possible only when the clinician has an accurate knowledge of the origin and nature of these cells. It is pertinent to state that these malignant cells releases certain biomarkers into the general circulation. Currently, screening for malignant tumors is done with various panels of biomarkers. Till date, there is no one serum biomarker capable of identifying the various lung cancer types. As such, accurate diagnosis is done only with immunohistochemistry and histological analysis of tumor biopsies. This article discusses the different protein biomarkers employed in the diagnosis of lung cancer and recent advances in uniform biomarker discovery.

**Keywords:** Biomarker, Lung Cancer, Morphology, Histology

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### INTRODUCTION

Lung cancer has a very high prevalence rate. It is the major cause of cancer-related mortality and morbidity globally. Studies have shown that at least 1.5-1.8 million cases of lung cancer are diagnosed each year. In 2012, 1.6 million deaths were attributed to this condition (Arya and Bhansali, 2011; Brambilla and Travis, 2014; Ferlay et al., 2015; Sung and Cho, 2018). Research shows that only 13-15 percent of cancer cases attain the 5-year survival rate (Fiorentino et al., 2011). The high mortality rate is attributed to late diagnosis of this condition. The basic explanation is metastases from the respiratory organ (the lung) into the central nervous system. This has been established in more than 50 percent of lung cancer cases (Cho et al., 2005; Gaspar et al., 1997). Thus, the key to reducing the high mortality rate is early detection & diagnosis. Accurate diagnosis, effective treatment, and development of highly effective medication is made possible by a first-hand knowledge of the pathophysiology of tumor development, and expression of biomarkers peculiar to lung cancer.

### HISTOLOGY OF LUNG CANCER

Cancer of the lung is a malignant tumor, one that may arise from the bronchioles, bronchial epithelium, bronchial mucous glands, and the alveoli. Features of the condition include post-treatment relapses, various histological differences, and metastases. The most recent classification of lung tumors was released by the World Health

Organization in 2015 (Schnabel and Junker, 2015). The major lung cancer types include small cell lung carcinoma (SCLC) and non-small-cell lung carcinoma (NSCLC). Clinical research has shown that majority of lung cancer cases (up to 80 percent) are of the NSCLC form (Schnabel and Junker, 2015, Travis, 2012). Non-small-cell carcinoma features diverse clinical forms such as: squamous cell carcinoma, large cell carcinoma, adenocarcinoma, large cell neuroendocrine carcinoma, and adenosquamous carcinoma. Adenocarcinoma originates from the bronchial mucosa, specifically the glandular cells. Currently, it represents the major histological subtype among the other lung cancer types. Bronchial epithelial cells serve as the origin for squamous lung cancer. The characteristics of squamous lung cancer include: keratinization, the presence of intercellular bridges or the formation of keratin pearl. Adenosquamous carcinoma contains both squamous and gland-like cells (Weynants et al., 1990). Large cell neuroendocrine carcinoma contains polygonal cells of large sizes. The polygonal cells do not show any evidence of histological differentiation. Of course they are malignant epithelial tumors. The tumor originates from the cells of the smooth muscles of the respiratory wall or from neuroendocrine cells of the respiratory tract. Large-cell carcinoma on the other hand are a heterogeneous group of malignant neoplasms that are devoid of architectural and cytologic features of small cell carcinoma, and squamous or glandular differentiation (Muller, 1984).

It is an established fact that both endogenous and exogenous factors contribute to the occurrence and development of cancer of the lung in each patient. Thus, cancer of lung, just like every oncological disorder, has a heterogeneous nature. Along with its many histological subtypes, lung cancer has pathological and molecular subtypes – characterized by epigenetic and cellular genetic changes and varying combinations of protein markers. Presently though, there is very limited data on protein biomarkers of the various histological sublung cancer types. However, some genetic studies presents the probability of certain genetic mutations. Intense studies have been conducted on mutations of epidermal growth factor receptors in lung adenocarcinoma. The study showed that the probability of EGFR mutations in lung adenocarcinoma patients was on the high side, increasing linearly from 3.7% to 18.5% (18-30 years and 81-100 years respectively). Studies also show that the probability of mutations in men is lower than that in female non-smokers (Dogan et al., 2012; Imyanitov et al., 2016). On the other hand, male non-smokers have a higher probability of EGFR mutation compared to smokers (Dogan et al., 2012).

There is a great need for accurate identification of lung cancer histology and their molecular subtype due to the various treatment strategies. Protein biomarkers released by each tumor cell plays an important role in carcinogenesis. The use of blood plasma in the determination of the nature and origin or malignant cells for diagnosis requires proper understanding about protein biomarker expression, their sensitivity and specificity, and their release by the various lung cancer cells (Capelozzi, 2009; Marshall et al., 2013; Vazquez et al., 2007).

## CURRENT METHODS OF DIAGNOSIS

Cancer detection is done mostly in the late stages. This is due to the various symptoms that characterize this stage. They include dyspnea, coughing up blood, coughing, and pains in the chest. Discovery of lung cancer in its early stage is mostly accidental. Diagnosis is done mostly by computed tomography and chest radiography. However, these methods can only identify visible and irreversible changes in the lung. Thus, there is a great need for more diagnostic methods. This challenge can be best overcome by the discovery of novel, very sensitive, and specific biomarkers (Marshall et al., 2013).

## CIRCULATING BIOMARKERS OF CARCINOGENESIS

The significance of protein biomarkers with respect to diagnosis of carcinogens, is determined by their specificity and sensitivity. The sensitivity of a particular biomarker is determined by the number of positive results gotten from analysis of a group of cancer patients. Conversely, biomarker specificity is determined the number of negative results gotten from analysis of both healthy people and patients with the benign form of the disease. Presently, researchers in the medical field have been unable to identify biomarkers with 100% specificity and sensitivity. Also, cancer-specific biomarkers have been found in the plasma of people without the disease.

Biomarkers of lung cancer may be detected in a non-invasive pattern through the use of biological materials like sputum, blood, tumor tissues, urine, and exhaled breath condensate. Exhaled breath condensate which originates from the respiratory tract has proteins, DNA, and cytokines in it (Rabinowits et al., 2009). A 2003 study by Mitas et al. has established a difference between the condensate of lung cancer patients and those of healthy people. However, there has been no detection of any specific biomarker. No specific markers have also been identified in sputum (Mitas et al., 2003).

It is pertinent to state that blood is the most important source of biomarker identification. This is understandable due to the ease with which cellular debris from the tumor penetrates the general circulation. As a result, the clinician may use blood as a liquid biopsy (minimally invasive of course). Blood contains RNAs, proteins, circulating lipids, DNAs,

and miRNAs, all associated with the tumor. It also contains endothelial, immune, stromal, and cancer cells (Chan et al., 2009). This explains why blood is seen as a complex matrix.

Biomarkers associated with tumors are biological molecules which act as indicators of pathogenic events or pharmacodynamics/pharmacological response to therapy (Jantus-Lewintre et al., 2012). Different cancer-biomarkers can be used to differentiate between physiological and pathological processes (Sung and Cho, 2018). The origin of an ideal biomarker may be traced to a neoplastic cell, is discernible in benign and healthy tissues. The biomarker may be identified in the biological fluids using very basic methods. It should be specific, not-expensive, and sensitive.

There are several types of tumor biomarkers – epigenetic biomarkers (alterations in DNA methylation profile), genetic biomarkers (expression of matrix RNA, mutations, and changes in the number of copies), metabolic biomarkers (changes in the spectrum and level of metabolites with low molecular weight), proteomic (changes in profile and level of protein expression), circulating RNAs and DNAs, exosomal miRNAs, endothelial, stromal, and immune cells, circulating tumor cells, and protein biomarkers (Rabinowits et al., 2009; Mitas et al., 2003; Chan et al., 2009; Jantus-Lewintre et al., 2012; Andre et al., 2002; Montani et al., 2015; Nagrath et al., 2007; Sozzi et al., 2014; Sozzi et al., 2003; Valenti et al., 2006). Generally, proteins are the most important biomarkers for diagnosis of lung cancer due to the role they play in cellular processes. Lung cancer screening is done using a panel of biomarkers (mostly cytokeratins; CYFRA 21-1), pro-gastrin-releasing peptide, carcinoembryonic antigen, and epithelial cell adhesion molecule. Practically though, this system does not provide valuable information and sufficient sensitivity for optimal screening. For instance, in lung cancer diagnosis, the specificity and sensitivity of carcinoembryonic antigen is 68 percent and 69 percent respectively. Cytokeratins, on the other hand, has a sensitivity of 43 percent, and specificity of 89 percent (Paci et al., 2010). A 1996 study by Kato showed that ProGRP (pro-gastrin-releasing peptide) had an 84% and 95% sensitivity and specificity respectively (Kato, 1996).

Biomarker discovery may be done using various approaches. Mass spectrometry analysis is mostly used for profiling of tumors.

## PROTEIN BIOMARKERS EMPLOYED IN THE DIAGNOSIS OF LUNG CANCER

Diagnosis of lung cancer may be done early, although this depends on the detection of autoantibodies and protein markers specific for each cancer type (Jett et al., 2014). 1613 patients were involved in a screening studies in the United States. The studies involving the use of EarlyCDT<sup>®</sup>-Lung test showed stage 1 lung cancer. The blood test had a higher specificity in detection of early lung cancer compared with imaging tests (Jett et al., 2014). Chapman et al. (2011) achieved a similar level of sensitivity and specificity for diagnosis of lung cancer using autoantibodies. It is therefore pertinent to state that high specificity and sensitivity levels makes the autoantibody panel indispensable in the early diagnosis of lung cancer.

Although great advances have been made in the discovery of biomarkers of lung cancer, we do not have any data on biomarkers with very high sensitivity and specificity. Several factors may contribute to this:

- The techniques employed in the search for biomarkers may be inefficient
- Tumors have a genetic heterogeneity
- Ineffective research design
- Laboratory tests are not reproducible
- Biomarkers analyzed have a very low concentration
- Inadequate number of tissue banks for screening (Sozzi et al., 2003).

That notwithstanding, there has been great advancement on lung cancer therapy, probably due to target treatment approaches. For a lung cancer therapy to be effective, one must have prior knowledge about specific molecular targets. This highlights the importance of lung cancer diagnosis, and identification of the histology of the cancer. Currently, several biomarkers are employed in the clinical setting for diagnosis of lung cancer.

The serum concentration of some biomarkers is on the low side, and as such, each biomarker cannot be used on its own for early diagnosis of lung cancer. The affected biomarkers are CYFRA 21-1, PrpGRP, and CEACAM (Mizuguchi et al., 2007; Pujol et al., 1993). Therefore, they must be used in combination. Adenocarcinoma may be detected with CYFRA 21-1 and CEACAM (Okada et al., 2004). Okada et al. found a significance difference between healthy people and lung cancer patients using a panel of CA125, CEACAM, NY-ESO, and CYFRA 21-1 (Goetsch, 2011). Neuron specific enolase, CYFRA 21-1, and CEACAM have been used by other authors for the differentiation of lung cancer histology (Doseeva, 2015).

Certain serum biomarkers have increased the accuracy of lung cancer diagnosis. These include C-reactive protein, lactate dehydrogenase, NSE, CEACAM, and CYFRA (Yu et al., 2014). Bigbee et al. suggests that early diagnosis of lung

cancer may be done with a panel of biomarkers including transthyretin, prolactin, selectin E, thrombospondin 1, plasminogen activator, tissue type, macrophage migration inhibitory factor, ERBB2, EGFR, serum APBA, and CYFRA 21-1, with a sensitivity of 77.1 percent, and a specificity of 76.2 percent (Bigbee et al., 2012).

A review of studies on early diagnosis of lung cancer shows that combining various tumor-linked biomarkers could be more effective than using each on its own (Mehan et al., 2012).

But that notwithstanding, the medical field is yet to find any composition for lung cancer detection (whether at the premalignant or early stages) (Jantus-Lewintre et al., 2013).

## BIOMARKERS OF DIFFERENT LUNG CANCER TYPES

Investigations are being carried out on new cancer-related markers alongside those currently used for the clinical diagnosis of lung cancer. For instance, the blood level of carcino embryonic antigen increases during a cancer attack. Carcino embryonic antigen is an established 180-kDa glycoprotein (Foa et al., 1999; Lee et al., 2014). Carcino embryonic antigen plays a very important role in cell adhesion processes (Kuespert et al., 2006). This explains the high metastatic potential of tumors with high CEA expression. CEA plays an important role in heterotypic and homotypic interactions with other cell (Pachter et al., 2003). High serum levels of CEA is associated with brain metastases (Reiber, 2001; Reiber et al., 1986). CEA serum levels plays an important role in determination of prognosis, and the risk of recurrence and death from cancer of the lung (Hotta et al., 2000; Grunnet and Sorensen, 2012). It is important to note that there is no correlation between the level of CEA and the stage of the disease (Pachter et al., 2003).

### Small cell lung cancer

The origin of small cell lung cancer is the neuroendocrine cell(s) of the amine precursor uptake and decarboxylation system (Stovold et al., 2012). SCLC exhibits two major biological features of the neuroendocrine cells of APUD system – production of NSE and L-DOPA decarboxylase. The gene L-DOPA decarboxylase encodes for the enzyme that catalyzes dopamine biosynthesis in humans (Papadopoulos et al., 2015). NSE is an enolase isoenzyme with two nearly-identical polypeptides (39-kDa) produced in the peripheral and central neurons of neuroectodermal origin. NSE is specific for small cell lung carcinoma (Yu et al., 2014). Other hormones produced in small cell lung cancer include estrogen, melanocyte-stimulating hormone, calcitonin, antidiuretic hormone, growth hormone, serotonin, and adrenocorticotrophic hormone.

Pro-gastrin-releasing peptide is another important biomarker of small cell lung cancer. Blood levels of ProGRP are high in patients with medullary thyroid cancer and small cell lung cancer ( $>200 \text{ pgmL}^{-1}$ ). The plasma level of ProGRP in healthy people is  $35 \text{ pgmL}^{-1}$  while that in benign diseases is  $45\text{-}10^3 \text{ pgmL}^{-1}$ . ProGRP has a higher specificity compared with NSE. However, its application in further studies is somewhat complicated because of its highly unstable nature and difficulty of identification.

### Adenocarcinoma

The glandular cells of the bronchial mucosa serves as the origin of adenocarcinoma.

Diagnosis depends on the identification of molecular markers of mutations, most especially ERCC, EGFR, KRAS, RRM1, EML4-A1k and TS (Sholl, 2015). The PSF3 protein (DNA replication complex GINS) has recently been identified as an important biomarker of adenocarcinoma (Tane et al., 2015; Tauchi et al., 2016; Hokka et al., 2013). PSF3 belongs to the GINS heterotetrameric complex comprising systemic RNA interference defective protein 5 (SLD5), GINS complex subunit 1 (PSF1), GINS complex subunit 2 (PSF2), and GINS complex subunit 3 (PSF3). This complex links to proteins. The proteins in turn regulate the initiation and progression phases of DNA replication (Bermudez et al., 2011). It is a fact that PSF3 is overexpressed in adenocarcinoma, implying that its plasma level should be higher. However, there is currently no data on the blood level of PSF3. Other novel lung adenocarcinoma-associated proteins include lamin (LMN), neutrophile defensing (DEF), vimentin (VIM), cytoplasmic actin (ACT), tubulin (TUB), clusterin (CLU), cathepsin D (CTSD), nucleolin (NCL), and dmucin-1 (MUC1). Studies have shown that adenocarcinoma diagnosis would be improved by identification of such proteins.

### Squamous lung cancer

Modified bronchial epithelial cells serve as the origin of squamous lung cancer. An important feature of squamous lung cancer is the increased level of CYFRA 21-1. This increase occurs during malignization of normal epithelial cells. CYFRA 21-1 has a high serum expression in patients with metastatic squamous lung cancer.

Squamous cell carcinoma antigen another protein specific for squamous lung cancer. The 48-kDa protein is highly expressed in squamous lung cancer (Lakshmanan, et al., 2015; Wang et al., 2010; Ferrigno et al., 1994). Squamous cell

carcinoma antigen inhibits serine proteases like cathepsin L, calpain 1, and chymotrypsin (Kato et al., 1985). It also act as an inhibitor of tumor cell apoptosis while also stimulating invasion and metastasis (Suminami et al., 1998).

### Adenosquamous carcinoma

Adenosquamous carcinoma shares the features of adenocarcinoma and squamous cell carcinoma. Its protein biomarker is mucin (MUC), also shared by both histotypes (Lakshmanan, et al., 2015).

### Large cell carcinoma

The major characteristics of large cell carcinomas are small groups of non-differentiated, dual-core or multi-core polymorphic cells (Muller, 1984). There's no data on the specific biomarkers of large cell carcinoma.

### Large cell neuroendocrine carcinoma

This is a very rare type of carcinoma. Diagnosis and treatment of large cell neuroendocrine carcinoma is somewhat difficult. Topoisomerase somatostatin precursor and excision repair 1, endonuclease non-catalytic subunit is overexpressed in large cell neuroendocrine carcinoma (Makino et al., 2016).

## CONCLUSION

Diagnosis of lung cancer is made difficult by the absence of sensitive and specific biomarkers. Of recent, panels of biomarkers have been applied in lung cancer detection. These biomarkers improve early detection of lung cancer. A biomarker panel consisting CEA, CYFRA21-1, MUC, ProGRP, PSF3, SST, and SCCA is recommended as it differentiates between the histological sublung cancer types. Also, the accuracy of diagnosis may be enhanced with aptamer-based identification of biomarkers of lung cancer, such as TUB, DEF, VIM, and LMN.

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