

Lung cancer biomarkers. A literature review

Biomarcadores de câncer de pulmão. Uma revisão de literatura

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ABSTRACT

Lung cancer is the first in terms of incidence and mortality, being responsible worldwide for about 1.8 million deaths. In Brazil 31,270 new cases were diagnosed in 2018, 18,740 in men and 12,350 in women. One of the main challenges about lung cancer is performing an early diagnosis, in most cases the disease is detected in the late stages, which implies in poor prognoses. Tumor biomarkers are hugely relevant in early diagnosis, understanding of carcinogenesis, prognostic determination and therapeutic choice. The present paper reviews non-small cell lung cancer biomarkers described in the literature and their diagnostic, prognostic and therapeutic applications, intervention and therapeutic control for individualized therapy. Although there is still a vast universe to be explored, studies reveal a promising future for lung cancer treatment with increasingly personalized and assertive therapies that increase the chances of progression-free survival.

Key words: lung neoplasms; biomarkers; tumor; humans.

RESUMO

O câncer de pulmão é o primeiro em incidência e mortalidade, sendo responsável mundialmente por cerca de 1,8 milhão de mortes. No Brasil, 31.270 casos novos foram diagnosticados em 2018, sendo 18.740 em homens e 12.350 em mulheres. Um dos principais desafios do câncer de pulmão é o diagnóstico precoce, na maioria das vezes a doença é detectada em fases tardias, o que implica em mau prognóstico. Os biomarcadores tumorais são extremamente relevantes no diagnóstico precoce, compreensão da carcinogênese, determinação do prognóstico e escolha terapêutica. O presente trabalho revisa biomarcadores de câncer de pulmão de células não pequenas descritos na literatura e suas aplicações diagnósticas, prognósticas e terapêuticas, intervenção e controle terapêutico para terapia individualizada. Embora ainda exista um vasto universo a ser explorado, estudos revelam um futuro promissor para o tratamento do câncer de pulmão com terapias cada vez mais personalizadas e assertivas que aumentam as chances de sobrevida livre de progressão.

Palavras-chave: neoplasias pulmonares; biomarcadores tumorais; humanos.

INTRODUCTION

According to the WHO, cancer is the leading cause of death in the world and is estimated to be responsible for 9.6 million deaths in 2018. Lung cancer (LC) is the first in terms of incidence and mortality, with approximately 2.1 million cases diagnosed in the

previous year, and due to its poor prognosis, was responsible for 1.8 million deaths worldwide⁽¹⁾.

In Brazil, an estimated 31,270 new cases were diagnosed in 2018, of these 18,740 in men and 12,350 in women. In 2017, the number of deaths from LC reached 27,833, representing the highest rate of death from cancer in men (15.9%), and the second

highest rate in women (11.4%), second only to breast cancer (16, 2%) which is more prevalent in this population^(2,3).

LC can be classified into two main types, according to the cells that initiate it, these being: non-small cell LC and small cell LC. Non-small cell LC is the most prevalent type and within this group are squamous cell carcinoma, large cell carcinoma and adenocarcinoma. Small cell LC is less common and tends to spread more rapidly than non-small cell LC. Most cases of LC are caused by smoking, however there are other factors that can influence the development of the disease, such as family history, secondhand smoke, radiation or occupational exposure to certain chemicals and pollutants⁽⁴⁾.

Diagnosis of this disease is performed through chest radiography in most cases, an easy to perform and relatively low-cost exam, but it can also be done through computed tomography, positron emission tomography, bronchoscopy and biopsy⁽⁵⁾. One of the main challenges in LC is to make an early diagnosis, in most cases the disease is detected already in its late stages, which implies a poor prognosis. Therefore, it is necessary to understand molecular biomarkers, which defined in a general and simplified way, are components that distinguish between the normal status and the abnormal status of a cell, thus aiding in the early diagnosis, the understanding of carcinogenesis, prognostic determination and the choice of therapy. To start LC treatment, it is necessary to assess the histological type of the cancer, its size and location, its stage and the general health conditions of the patient. The choice of therapy is individualized for each patient and this is not always a simple task, the decision process must count on the participation of a multidisciplinary team together with the patient⁽⁶⁾.

The range of treatments includes surgery, chemotherapy, radiotherapy, target therapy and/or a combination of these modalities, depending on the type of cancer and how advanced the stage is. Surgeries can be of three types, segmentectomy and wedge resection (when a small part of the lung is removed), lobectomy (the entire lung lobe affected by the tumor is removed) and pneumectomy (complete removal of the lung).

Taking into account the aforementioned information, the present work reviews the main biomarkers of non-small cell LC and their applications in diagnosis, prognosis and intervention, in addition to therapeutic control for individualized therapies.

MATERIAL AND METHODS

This bibliographic review seeks to understand the role of biomarkers in the population of patients with non-small cell LC, as they can assist in early diagnosis, in assessing disease progression,

in choosing the therapeutic intervention and what to expect in the future.

To elucidate the questions in this review, the electronic database PubMed was used, which comprises more than 29 million citations from MEDLINE's biomedical literature, life science journals and online books. The research was carried out in April/2019 and research carried out in humans and published in the past 5 years (2014-2019) in Portuguese or English were selected.

The terms used as research descriptors were: "biomarkers", "non-small lung cancer" and "humans". The search followed the MeSH (Medical Subject Headings) strategy, resulting in the following headings: (("Biomarkers" [Mesh]) AND "Carcinoma, Non-Small-Cell Lung / diagnosis" [Mesh]) AND "Humans" [Mesh].

The resulting studies were submitted to the following inclusion criteria: clinical studies involving humans, published in the past 5 years, in English or Portuguese, with abstract and full texts. Review studies, meta-analyses, editorials, letters, case studies and clinical studies in animals were excluded.

As a result of the research, 1,317 studies were obtained. After applying the inclusion criteria, 17 studies were included, one of which was in French, at the end of the process 16 studies were selected. After the selection process, the abstracts were read and studies that were not related to biological biomarkers were excluded, totaling the number of included studies to 8.

The selected articles are organized in the Table 1 below according to the author, year of publication, aim, methodology, outcomes and whether or not there was funding.

EGFR (Epidermal Growth Factor Receptor) Mutation

The epidermal growth factor (EGFR) receptor, also known as: ERBB; HER1; mENA; ERBB1; ERBB2; PIG61; NISBD2, encodes a transmembrane glycoprotein of the protein kinase family. This protein is a cell surface receptor, which binds to the epidermal growth factor, and this binding induces the dimerization of the receptor and the autophosphorylation of the tyrosine kinase domain, leading to the activation of signaling cascades for cell growth, differentiation, migration, proliferation and apoptosis⁽⁷⁾.

The EGFR coding gene is located in the short arm of chromosome 7 (7p11.2), the EGFR transmembrane protein is composed of 28 exons and three domains: N-terminal extracellular domain, lipophilic domain and the intracellular tyrosine kinase domain C-terminal and encodes a protein of 1210 amino acids^(7,8).

EGFR is usually overexpressed in many types of epithelial

TABLE 1-Summary table of selected articles

Author/year	Aim	Methodology	Outcomes	Funding
Marchetti A, et al -2014	To investigate the feasibility of detecting EGFR mutations in circulating tumor cells of patients with non-small cell LC by coupling the CellSearch system with next generation sequencing (NGS) in the 454 GS Junior system (454 Life Sciences, Branford, CT and Roche Applied Sciences, Indianapolis, IN).	Blood samples were collected from 37 patients participating in the TRIGGER study, a prospective multicenter phase II trial of erlotinib in patients with advanced non-small cell LC with EGFR-activating mutations in tumor tissue. 10 circulating tumor cells (CTC) were prepared from breast cancer patients without EGFR mutations in their primary tumors and 12 samples from healthy subjects were analyzed as negative controls. The CTC preparations, obtained with the VeridexCellSearch System, were subjected to the latest generation ultra-deep sequencing (NGS) in the 454 GS junior platform from Roche.	The Cell Search system, together with NGS, has been reported to be highly sensitive and specific as a diagnostic tool for the analysis of EGFR mutations in CTC preparations, with potential clinical impact, and may be particularly useful in cases with a very limited amount of biological material or for monitoring the mutational status of the tumor during treatment, with special emphasis on the presence of mutations involved in the acquisition of resistance to TKIs.	Roche Pharmaceutical Industry
Sun M, et al - 2014	To explore the pattern of SPRY4-IT1 expression in non-small cell LC (NSCLC) tissues and cell lines, and investigate the effects of SPRY4-IT1 expression on NSCLC cell phenotypes both in vitro and in vivo.	SPRY4-IT1 expression was investigated in 121 paired NSCLC samples and histologically normal adjacent tissues using PCR (qPCR)	SPRY4-IT1 expression was down-regulated and correlated with a poor prognosis of non-small cell LC.	National Natural Scientific Foundation of China
Yamamoto S, et al - 2014	To provide a radiogenomic characterization of computed tomography of ALK-rearranged NSCLC (ALK + NSCLC) from data in a multi-institutional cohort.	In this retrospective study, tomographic studies, ALK status and clinical-pathological data from 172 patients with NSCLC from three institutions were analyzed. Twenty-four CT features plus six clinical-pathological covariates were used to identify a radiogenomic predictor of ALK+ status. This predictor was then validated in an independent cohort (n=113). Test analyses for precision and subsets were performed. A similar analysis was performed to identify a biomarker associated with lower progression-free survival (PFS) after therapy with the ALK inhibitor crizotinib.	ALK + NSCLC has distinct characteristics in the computed tomography image that, when combined with clinical covariates, discriminate ALK+ from non-ALK tumors and can potentially identify patients with a shorter durable response to crizotinib.	Not declared.
Bar J, et al - 2015	To evaluate the prognostic and predictive significance of serum levels of angiotensin-converting enzyme (ACE) and aldosterone, regulators of blood pressure, in patients with advanced non-small cell lung cancer (NSCLC) participating in the NCIC Clinical Trial Group Trial BR.24.	Angiotensin-converting enzyme and aldosterone were retrospectively measured using enzyme-linked immunosorbent assays at the start and during treatment, in serum samples from 226 and 176 of 296 participants, respectively. Cox regression was performed to correlate biomarkers and patient characteristics with overall survival (OS) and progression-free survival (PFS).	Low initial levels of ACE were predictors of a low total survival rate and predictive of the overall survival benefit of cediranib. An increase in the level of aldosterone with treatment may also be predictive of the overall survival benefit of cediranib. These biomarkers must be validated in additional anti-angiogenic assays in NSCLC and other cancers.	Ottawa Regional Cancer Center and Ottawa Regional Cancer Foundation

Chen X, et al - 2015	To investigate the diagnostic performance of folate-receptor positive circulating tumor cell in distinguishing non-small cell LC (NSCLC) from benign lung disease, a new polymerase chain reaction (PCR) technique targeting ligand detection.	Circulating tumor cells from 3 mL of blood were enriched by leukocyte immunomagnetic depletion and then labeled with a conjugate of a tumor-specific folic acid and a synthesized oligonucleotide. After washing the free conjugates, the removed ligand conjugates were analyzed by quantitative PCR.	The ligand-targeted PCR technique was feasible and reliable for detecting folate receptor-positive circulating tumor cells in patients with NSCLC, and circulating tumor cell levels could be used as a useful biomarker for the diagnosis of NSCLC.	This research was partially supported by the Guide Project of the Science and Technology Commission of Shanghai Municipality (grant 124119a8000); the Forefront and Emerging Technology Projects of Shanghai Shengkang Hospital Development Center (grant SHDC12013102); and the key project supported by Shanghai Science and Technology Commission (grant 13441902200).
Shimizu T, et al - 2016	The aim of this study is to determine whether the number of TYMS gene copies predicts the outcome in patients receiving pemetrexede (PMT). The association between the number of copies of the TYMS gene and the therapeutic efficacy of PMT plus carboplatin (CBDCA) in patients with advanced NSCLC was investigated in a phase II study.	The participants were patients who had never undergone treatment with chemotherapy, with advanced non-small cell LC, treated with pemetrexed plus carboplatin (CBDCA), a prospective phase II clinical study. TYMS (Thymidylate synthase) expression was assessed in 40 patients by gene copy number and protein expression using FISH and IHC. Therapeutic efficacy was assessed by investigating response rate (RR), disease control rate (DCR), progression free survival (PFS) and overall survival (OS).	The analysis of the number of TYMS gene copies is more adequate than TYMS protein expression for evaluation of TYMS expression. Amplification of the TYMS gene predicts outcomes for patients with NSCLC who receive pemetrexede.	Not declared
Niemeijer AN, et al - 2018	To show that PD-L1 and PD-1 tumor expression can be non-invasively quantified using PET-CT in patients with non-small cell LC.	Participating patients underwent full body scans for PD- (L) 1 using PET-CT. Thirteen patients were included in this exploratory of an open, single-center, one-arm biomarker study, the first in humans.	PD-L1 and PD-1 tumor expression can be non-invasively quantified using PET-CT in patients with non-small cell LC. Entire body PD-(L) 1 PET-CT reveals significant heterogeneity in the uptake of the tumor tracer both between patients and between different tumor lesions within patients.	Bristol-Myers Squibb (BMS)
Villalobos M, et al - 2018	The aim of this study was to assess the impact of ERCC1 on the survival of patients with non-squamous NSCLC (NS-NSCLC) stage IIIB / IV participating in the INNOVATIONS trial, treated with erlotinib / bevacizumab (EB) or cisplatin / gemcitabine / bevacizumab (PGB).	Retrospective analysis of the tumor tissue of 72 patients using immunohistochemistry to evaluate ERCC1 expression. The distribution between treatment arms was equal (36 patients in each). Two different H scores were calculated and correlated with survival.	The findings support the hypothesis that patients whose tumors have low ERCC1 expression benefit from cisplatin-based chemotherapy. In patients treated with erlotinib and bevacizumab, a positive effect on progression free survival was found for ERCC1 positive tumors based on the H score, but not with the modified scoring system.	Roche Pharmaceutical Industry

cancer, such as in non-small cell LC. Mutations in the EGFR gene, for example, the deletion in exon 19 or exon 21 (L858R), are considered activators and are predictive of good prognosis in therapies with tyrosine kinase inhibitors (erlotinib and gefitinib), others, such as an exon 20 mutation (T790M), usually appear after sometime during treatment and characterize resistance to therapy. Both mutations are considered pharmacogenetic biomarkers in non-small cell LC and help predict treatment outcomes⁽⁷⁾.

The peripheral blood of individuals with cancer contains free DNA molecules from tumor cells, also known as circulating tumor cells (CTCs). CTCs have been shown to be detectable in patients with advanced non-small cell LC⁽⁹⁾.

A research group evaluated EGFR-activating mutations from CTC preparations, extracted from blood samples from patients with non-small cell LC. CTC preparations were obtained by the Veridex Cell Search System and submitted to the latest generation ultra-deep sequencing (NGS) in the 454 GS junior platform from Roche⁽¹⁰⁾.

The possibility of detecting genetic mutations in CTCs instead of conventional tissue biopsy has some advantages: First, blood samples can be more easily and repeatedly obtained whereas tissue biopsies are invasive procedures and re-biopsies are challenging; Second, CTC scan represent the current state of neoplastic growth and are important in monitoring recurrences and in the development of tumor resistance; Third, CTCs can represent the entire neoplastic process (primary tumor/metastasis), while biopsy in a single location cannot reflect the various tumor sites.

The study demonstrated for the first time that it is possible to detect EGFR mutations through state-of-the-art ultra-deep sequencing from CTC preparations and the new diagnostic approach could be particularly useful in cases with limited amounts of biological material or to evaluate the mutational status of the tumor during treatment, especially in mutations developed in the acquisition of resistance to tyrosine kinase inhibiting therapies.

SPRY4-IT1 Expression in Long Non-coding RNAs

Although the human genome is 75% transcribed, a large fraction of this genome is non-coding, and produces long non-coding RNAs called lncRNAs, only 2% of the genome encodes proteins⁽¹¹⁾.

These lncRNAs participate in several biological processes, including modulation of apoptosis, invasion and reprogramming of stem cell pluripotentiality and parental imprinting, for these reasons, they are of great interest in oncological diseases⁽¹²⁾.

The SPRY4-IT1 lncRNA is an intronic derivative within SPRY4 (pulverized RTK signaling antagonist 4), which is a protein coding gene located in chromosome 5, widely expressed in the lung, adrenal tissue and others⁽¹³⁾.

The SUN et al., 2014 research group explored SPRY4-IT1 expression in NSCLC tissues and cell lines and what effects this expression had on NSCLC cell phenotypes both *in vitro* and *in vivo*⁽¹³⁾. To assess whether SPRY4-IT1 expression would affect tumorigenesis, cells of the SPC-A1 lineage (pulmonary adenocarcinoma cells) containing pCDNA-SPRY4-IT1 and empty vectors (SPC-A1 cells only) were inoculated into female mice. Eighteen days after injection tumors formed in the pCDNA-SPRY4-IT1 group were found to be smaller than those obtained from the control group.

The study demonstrated that the low SPRY4-IT1 expression is related to poor prognosis in patients with NSCLC, with low survival rates and high risk of metastases. On the other hand, high SPRY4-IT1 expression has been shown to inhibit the proliferation, migration and invasion of NSCLC cells and induce apoptosis.

These findings are important in understanding the pathogenesis of NSCLC and in directing targets for new therapies and for diagnosis, suggesting that SPRY4-IT1 could be a biomarker for poor prognosis in NSCLC.

Radiogenomic ALK (Anaplastic Lymphoma Kinase) gene

Located in chromosome 2, this gene encodes a tyrosine kinase receptor. This protein is composed of an extracellular domain, a hydrophobic elongation that corresponds to a transmembrane passage region, and an intracellular kinase domain. It is of great importance in brain development and exerts its effects on specific neurons in the nervous system.

This gene was found to have been rearranged, mutated or amplified in a number of tumors, including non-small cell LC, which represent up to 5% of all primary diagnoses and tend to occur in children and in patients without a history of smoking or ex-light smokers (10 packs/year). Chromosomal rearrangements are the most common genetic alterations in this gene and result in the creation of multiple fusion genes in tumorigenesis (known as EML4-ALK translocation)^(14,15).

Yamamoto et al., 2014 research group evaluated a way to characterize the ALK radiophenotype using computed tomography. A quantitative score was created, with the creation and validation of this score occurring in two stages: the first consisted of defining a radiogenomic biomarker of the ALK state in computed tomography in a training set, the second phase consisted of

validating this biomarker in an independent set of patients⁽¹⁶⁾. A total of 172 patients were included in this study, 47 ALK+, 65 with EGFR mutation, 41 with wildtype EGFR, 6 with KRAS mutation and 13 with TP53 mutation. Four characteristics were selected as predictive of ALK+ status, these being: central tumor location, pleural effusion, absence of pleural tail sign and age under 60 years.

The advantages of using an image score to identify the ALK+ phenotype in computed tomography data is, in addition to the ease of calculation, evidenced by the interobserver reliability tested in the study, it is easily detected in almost all patients through routine clinical evaluation, without being invasive.

The purpose of using the image score is not to replace the molecular test, but rather to provide radiologists with a tool to better understand the distinctive findings associated with ALK+ tumors and to raise clinical suspicion for this molecular subtype when appropriate through a more profound use of clinical imaging information associated with clinical covariates. In addition to discriminating ALK+ tumors from non-ALK tumors, an image biomarker can also identify patients with a short duration of response to ALK inhibitor therapy (crizotinib), thus aiding in the determination of the stage.

Serum Angiotensin-Converting Enzyme (ACE) and Aldosterone Levels

The renin-angiotensin-aldosterone system (RAAS) is one of the main regulators of blood pressure and is also implicated as a potential regulator of angiogenesis^(17,18).

Angiotensin II formed by the angiotensin-converting enzyme (ACE) activates AT1 and AT2 receptors, with the activation of the AT1 receptor increasing endothelial production of reactive oxygen species through adenine and nicotinamide dinucleotide phosphate (NADP), reducing localized nitric oxide levels and causing endothelial dysfunction. In addition, the activation of AT1 can induce tissue fibrosis in some situations and increase interstitial tissue pressure (ITP) in affected organs. This endothelial dysfunction and increased ITP can limit the delivery of nutrients by the vascular system⁽¹⁷⁻²⁵⁾.

Bar et al., 2015, tested the hypothesis that high RAAS activity indicates an unfavorable environment for angiogenesis, along with endothelial dysfunction and increased ITP, and therefore, may be related to a favorable prognosis and/or inhibition of the signaling pathway of vascular endothelial growth factor, a promising therapeutic target for NSCLC cells, one of these inhibitors being cediranib[®]. ACE and aldosterone were chosen because they are relatively stable in serum samples⁽²⁶⁾. The results of the study

supported the hypothesis that greater RAAS activity is associated with a good prognosis, however high activity was inversely correlated with antiangiogenic efficacy. Patients with baseline ACE levels (less than or equal to 115 ng/mL) benefited from the combination of cediranib[®] + chemotherapy, and increased levels of aldosterone (above 250 pg/mL) during treatment with cediranib revealed a positive prognosis and is possibly a good marker of the efficiency of antiangiogenic treatment. Low serum ACE levels (<115 ng/mL) were predictive of improvement in the overall survival rate after treatment with cediranib[®].

The role of serum ACE and aldosterone levels as predictive and prognostic biomarkers for antiangiogenic treatments needs to be further explored in future studies.

Folate Receptor-Positive Circulating Tumor Cells

Folate receptors (FRs) are cell surface glycoproteins, highly expressed in NSCLC (approximately 72% to 83% over express FR on the cell surface)^(27,28).

The Chen et al., 2015 research group tested folate-receptor positive performance in circulating tumor cells in order to distinguish patients with NSCLC from those with benign lung diseases (pneumonia, pulmonary tuberculosis, bronchiectasis or pneumothorax), using ligand-targeted polymerase chain reaction (LT-PCR) technique. In addition, diagnostic yields between CTCs and other tumor markers (carcinoembryonic antigen [CEA], neuron specific enolase [NSE] and Cyfra 21-1) in patients with NSCLC were compared⁽²⁹⁾. This study included 756 participants of which 56 were healthy volunteers, 227 had benign lung disease and 473 initially diagnosed with NSCLC. The specificity and sensitivity of the CTC diagnostic model in combination with tumor markers and only with tumor markers (CEA in combination with NSE and Cyfra 21-1) were tested using binary logistic regression analysis to determine whether CTC can improve the accuracy of diagnosis.

Based on the receiver operator characteristic (ROC) curve, the ideal cut-off point for differentiating patients with NSCLC and benign lung disease was 8.93 units, with a sensitivity of 74.4% and specificity of 86.6%. Patients with NSCLC showed a curve value of 12.41 ± 9.02 units, a significantly higher number than patients with benign lung disease with 6.95 ± 5.45 units and healthy patients with 5.95 ± 4.57 units.

The LT-PCR technique used in this study was based on negative leukocyte depletion followed by labeling with folate-linked oligonucleotide and quantification with quantitative PCR. The study demonstrated that CTC levels in NSCLC patients are increased when compared to patients with benign lung diseases and healthy volunteers.

The LT-PCR technique proved to be viable and reliable in the detection of folate-positive CTCs in patients with NSCLC, CTC levels can be used as biomarkers in the diagnosis of NSCLC, especially when combined with other serum tumor markers. Further studies are needed to investigate the prognostic or “liquid biopsy” value of CTCs when detected by the LT-PCR method in patients with NSCLC, however results are promising.

Thymidylate Synthase (TYMS)

Thymidylate synthase (TYMS) is a folate-dependent enzyme that is involved in DNA synthesis, DNA repair and cancer cell proliferation^(30,31).

Pemetrexed is an antifolate antineoplastic agent that selectively and potently inhibits TYMS, this being the main therapeutic target of the drug. Overexpression of TYMS correlates with resistance and decreased sensitivity to pemetrexed in cancer cell lines.

Shimizu et al., 2016 research group assessed how the number of copies of the TYMS gene predicts the outcomes of pemetrexed therapy and how it interferes with the therapeutic efficacy of the combination of pemetrexed with carboplatin in patients with NSCLC in an open phase II clinical trial⁽³²⁾.

Forty patients with advanced NSCLC (stage IIIB or stage IV), who had never undergone chemotherapy, with ages between 20 and 75 years, were evaluated for TYMS (gene and protein) expression in cancerous tissue. The patients were separated into two groups according to the number of TYMS gene copies: the amplified TYMS group and the non-amplified TYMS group. Evaluating the rates of response, disease control, progression-free survival and overall survival, the non-amplified TYMS group performed better than the group with amplified TYMS. No patient in the amplified TYMS group achieved a complete or partial response to therapy. TYMS protein expression was not significantly correlated with the number of copies of the TYMS gene.

The results showed that the number of copies of the TYMS gene is a better predictive biomarker for the response to pemetrexed therapy than TYMS protein expression. Amplification of the TYMS gene can be measured in LC tissue at low cost, using the fluorescence in situ hybridization (FISH) technique, which in addition to being more sensitive and specific, is apparently more suitable in clinical practice when compared to mRNA and TYMS protein expression⁽³²⁾.

In summary, the analysis of the number of copies of the TYMS gene proved to be a useful method for the evaluation of TYMS expression in LC tissue and the amplification of the TYMS gene is a predictor of outcomes in pemetrexed therapy.

PD-1 and PD-L1 expression

The programmed cell death receptor-1 (PD-1) is expressed on the cell surface of activated T cells. PD-L1 and PD-L2 are ligands for the PD-1 receptor and are commonly expressed on the surface of dendritic cells or macrophages.

Both the receptor and the ligands belong to the family of immune checkpoint proteins and act as co-inhibitors that can inhibit or limit the response of T cells. The PD-1/PD-L1 system ensures that the immune system is activated only at the right moment, in order to minimize chronic autoimmune inflammations^(33,34).

PD-L1 expression, measured by immunohistochemistry, is related to the rate of progression-free survival after therapy with monoclonal antibodies. Approximately 10% of patients whose cells do not express PD-L1, detected by immunohistochemistry, have a favorable response to PD-(L) 1 therapy. This fact can be explained due to the heterogeneity of PD-L1 expression by small cell lung tumors⁽³⁴⁾.

Controversies in PD-L1 expression in tumors, detected using immunohistochemistry, question whether this could be a good biomarker for therapies with monoclonal antibodies.

Niemeijer et al., 2018, quantified PD-1 PD-L1 expression in patients with NSCLC, using whole body positron emission tomography (PET Scan), a non-invasive technique, using the radiotracers 18F-BMS-986192 and 89Zr-nivolumab. The study demonstrated that the use of PET Scan with the radiotracers 18F-BMS-986192 and 89Zr-nivolumab, is viable and safe in the quantification of PD-1 and PD-L1. This implies that these markers could be used to quantify the expression of PD-(L) 1 non-invasively in future immunotherapeutic studies. As this is a small study, larger data sets are needed to validate these results⁽³⁴⁾.

ERCC1 expression

The excision repair cross-complementation group 1 (ERCC1) protein is involved in the nucleotide excision repair pathway and is necessary for repairing DNA lesions. O gene codificante localizado no cromossomo 19q, é expresso em todos os tecidos em níveis relativamente elevados. The coding gene, located in chromosome 19q, is expressed in all tissues at relatively high levels⁽³⁵⁾.

Studies have demonstrated a correlation between ERCC1 levels and the results of platinum-based chemotherapy in patients with NSCLC. Villalobos et al., 2018 conducted a study to assess the impact of ERCC1 levels on the survival of patients with advanced non-squamous (stages IIB/IV) NSCLC participating in the INNOVATIONS trial (open, randomized multicenter phase II

study), who received erlotinib/bevacizumab (EB) or cisplatin/gemcitabine/bevacizumab (CGB) as treatment. A total of 72 patients were assessed, equally distributed in each treatment arm (36 patients), the immunohistochemistry technique was used to evaluate ERCC1 expression and two scores: H and modified H were calculated and related to the survival rate⁽³⁶⁾.

The percentage of positive tumor nuclei was calculated for each sample and a proportional score was assigned (0 if 0%, 0.1 if 1-9%, 0.5 if 10-49% and 1.0 if 50% or more) this proportion was multiplied by the nucleus staining intensity to obtain a final semi-quantitative H score, in the modified H score the H score was additionally calculated using the usual formula: $[1 \times (\% \text{ cells } 1+) + 2 \times (\% \text{ cells } 2+) + 3 \times (\% \text{ } 3+ \text{ cells})]$. The median values of all H/modified H scores were chosen as the cutoff points for the separation between ERCC1-positive tumors from ERCC1-negative tumors⁽³⁶⁾.

The analyses performed showed longer progression-free survival in patients with negative ERCC1 treated with CGB (in both scores), which supports the hypothesis that patients with low levels of ERCC1 who have a poor prognosis can be treated with cisplatin-based chemotherapy to obtain better results. In patients treated with erlotinib and bevacizumab, a positive effect on progression-free survival was found for ERCC1 positive tumors based on the H score, but not on the modified H score.

The inconsistent results between the two scoring systems, according to the authors, underscore the importance of an international consensus to demand a homogeneous evaluation methodology.

DISCUSSION

In this review it was possible to note that the mechanism of lung carcinogenesis is not yet fully elucidated, although it has been widely studied. There are still numerous contradictions.

Biomarkers can be classified into several categories: diagnostic, stage prediction, prognostic and therapeutic choice/evolution. Notably, there is a large niche of studies on the early diagnosis of NSCLC, on the other hand, studies on the evaluation and prediction of therapeutic response have grown exponentially^(10,15).

CTCs are promising cells for the diagnosis of NSCLC, known to be expressed in blood from solid tumors. They have an important role in the development of metastases and improvements in CTC detection techniques, such as the Cell Search system associated with NGS, have shown great potential as “liquid biopsy”, especially in LC where tissue accessibility is often a challenge, and have value

in the assessment of both diagnostic and therapeutic efficacy.

Auxiliary biomarkers are necessary in imaging diagnostics, since there is a high rate of false positives in the screening process by computed tomography, which leads to multiple rounds of screening, radiation exposure, and increased time and costs. Therefore, the development of complementary non-invasive biomarkers can be of great use for better staging of the patient. In the case of the ALK state radiogenomic biomarker, presented in this review, there is also its utility in discriminating the best choice of therapy to be employed in each case (ALK+ and non-ALK)⁽¹⁶⁾.

The evaluation of the expression of ACE and aldosterone, thymidylate synthase, PD-1/PD-L1 and ERCC1 proved to be important in the analysis of therapeutic efficacy and are in line with the personalized therapy strategy for combating NSCLC.

The innovative approach to quantify SPRY4-IT1 expression in long non-coding RNA played a relevant role in the understanding of lung carcinogenesis and in the analysis of prognosis, therefore being a considerable target for future studies⁽¹³⁾.

CONCLUSION

Currently, only 16% of cancers are diagnosed at an early stage (localized cancer), when there are higher chances of treatment. Improvements in diagnosis facilitate the early detection of the disease and direct toward an effective intervention, these being necessary measures to reduce mortality rates due to LC.

Among the biomarkers presented in this review, folate receptors in circulating tumor cells, accessed through peripheral blood, was efficient in differentiating between benign lung diseases and NSCLC. The other biomarkers presented here showed effectiveness in evaluating the therapeutic response, aiding in prognostic prediction and directing toward the best intervention. SPRY4-IT1 expression in long non-coding RNA needs to be further studied both as a therapeutic target and as a prognostic predictor.

Despite some biases present in the studies, such as insufficient number of patients, sometimes controversial conclusions, conflict of interest or lack of randomization, they represent the start of a new era and reveal a promising future for the treatment of LC, with increasingly more personalized and accurate therapies, which increase the chances of progression-free survival. There is still a vast universe to be unraveled and carrying out new studies that confirm the findings described here and further explore lung carcinogenesis of non-small cells are necessary.


Conflict of Interest: All authors disclaim any conflict of interest.

REFERENCES

1. Nasim F, Sabath BF, Eapen GA. Lung Cancer. *Med Clin North Am*. 2019; 103(3): 463-73.
2. De Sa VK, Coelho JC, Capelozzi VL, et al. Lung Cancer in Brazil: Epidemiology and Treatment Challenges. *Lung Cancer (Auckl)*. 2016; 7: 141-8.
3. Araujo LH, Baldotto C, De Castro Jr G, et al. Lung Cancer in Brazil. *J Bras Pneumol*. 2018; 44(1): 55-64.
4. Torre LA, Siegel RL, Jemal A. Lung Cancer Statistics. *Adv Exp Med Biol*. 2016; 893: 1-19.
5. Collins LG, Haines C, Perkel R, et al. Lung cancer: Diagnosis And Management. *Am Fam Physician*. 2007; 75(1): 56-63.
6. Lemjabbar-Alaoui H, Hassan OU, Yang YW, et al. Lung cancer: Biology and treatment options. *Biochim Biophys Acta*. 2015; 1856(2): 189-210.
7. Jurišić V, Obradovic J, Pavlović S, et al. Epidermal Growth Factor Receptor Gene in Non-Small-Cell Lung Cancer: The Importance of Promoter Polymorphism Investigation. *Anal Cell Pathol (Amst)*. 2018: 6192187.
8. Hodoglugil U, Carrillo MW, Hebert JM, et al. PharmGKB summary: very important Pharmacogene information for the Epidermal growth factor receptor. *Pharmacogenet Genomics*. 2013; 23(11): 636-42.
9. Krebs MG, Sloane R, Priest L, et al. Evaluation and Prognostic significance of Circulating Tumor cells in patients with non-small-cell Lung cancer. *J Clin Oncol*. 2011; 29(12): 1556-63.
10. Marchetti A, Grammasro MD, Felicioni L, et al. Assessment of EGFR mutations in circulating tumor cell preparations from NSCLC patients by next generation sequencing: toward a real-time liquid biopsy for treatment. *PLoS One*. 2014; 9(8): e103883.
11. Mekahli D, Bultynck G, De Smedt H, et al. Endoplasmic-reticulum Calcium depletion and disease. *Cold Spring Harb Perspect Biol*. 2011; 3(6): a004317.
12. Fatica A, Bozzoni I. Long non-coding RNAs: new players in cell differentiation and development. *Nat Rev Genet*. 2014; 15(1): 7-21.
13. Sun M, Liu XH, Lu KH, et al. EZH2-mediated epigenetic suppression of long noncoding RNA SPRY4-IT1 promotes NSCLC Cell proliferation and Metastasis by affecting the Epithelial-Mesenchymal transition. *Cell Death Dis*. 2014; 5: e1298.
14. Hallberg B, Palmer RH. Mechanistic insight into ALK receptor tyrosine kinase in human cancer biology. *Nat Rev Cancer*. 2013; 13(10): 685-700.
15. Hallberg B, Palmer RH. The Role of the ALK receptor in Cancer biology. *Ann Oncol*. 2016; 27(3): iii4-iii15.
16. Yamamoto S, Korn RL, Oklu R, et al. ALK molecular phenotype in non-small cell lung cancer: CT radiogenomic characterization. *Radiol*. 2014; 272(2): 568-76.
17. Ebrahimian TG, Tamarat R, Clergue M, et al. Dual effect of angiotensin-converting enzyme inhibition on angiogenesis in type 1 diabetic Mice. *Arterioscler Thromb Vasc Biol*. 2005; 25(1): 65-70.
18. Fraccarollo D, Galuppo P, Schraut S, et al. Immediate Mineralocorticoid receptor blockade improves myocardial infarct healing by modulation of the Inflammatory response. *Hypertension*. 2008; 51(4): 905-14.
19. Jain RK. Vascular and interstitial barriers to delivery of Therapeutic agents in tumors. *Cancer Metastasis Rev*. 1990; 9(3): 253-66.
20. Prud'homme GJ. Pathobiology of transforming growth factor beta in Cancer, Fibrosis and Immunologic disease, and therapeutic considerations. *Lab Invest*. 2007; 87(11): 1077-91.
21. Suda O, Tsutsui M, Morishita T, et al. Asymmetric dimethylarginine produces vascular lesions in endothelial nitric oxide synthase-deficient mice: involvement of renin-angiotensin system and oxidative stress. *Arterioscler Thromb Vasc Biol*. 2004; 24(9): 1682-8.
22. Van der Knaap R, Siemes C, Coebergh JWW, et al. Renin-angiotensin system inhibitors, angiotensin I-converting enzyme gene insertion/deletion polymorphism, and cancer: the Rotterdam Study. *Cancer*. 2008; 112(4): 748-57.
23. Veresh Z, Debreczeni B, Hamar J, et al. Asymmetric dimethylarginine reduces nitric oxide donor-mediated dilation of arterioles by activating the vascular renin-angiotensin system and reactive oxygen species. *J Vasc Res*. 2012; 49(4): 363-72.
24. Warnholtz A, Nickenig G, Schulz E, et al. Increased NADH-oxidase-mediated superoxide production in the early stages of atherosclerosis: evidence for involvement of the Renin-angiotensin system. *Circ*. 1999; 99(15): 2027-33.
25. Williams JS, Williams GH. 50th Anniversary of Aldosterone. *J Clin Endocrinol Metab*. 2003; 88(6): 2364-72.
26. Bar J, Ding K, Zhao H, et al. Angiotensin-Converting Enzyme and Aldosterone Serum Levels as Prognostic and Predictive Biomarkers for Cediranib in NCIC Clinical Trials Group Study BR.24. *Clin Lung Can*. 2015; 16(6): 189-201.
27. Christoph DC, Asuncion BR, Hassan B, et al. Significance of folate receptor alpha and thymidylate synthase protein expression in patients with non-small-cell lung cancer treated with pemetrexed. *J Thorac Oncol*. 2013; 8(1): 19-30.
28. O'Shannessy DJ, Yu G, Smale R, et al. Folate receptor alpha expression in lung cancer: Diagnostic and Prognostic significance. *Oncotar*. 2012; 3(4): 414-25.
29. Chen X, Zhou F, Li X, et al. Folate Receptor-Positive Circulating Tumor Cell Detected by LT-PCR-Based Method as a Diagnostic Biomarker for Non-Small-Cell Lung Cancer. *J Thorac Oncol*. 2015; 10(8): 1163-71.

30. Ferguson PJ, Collins O, Dean NM, et al. Antisense down-regulation of thymidylate synthase to suppress growth and enhance cytotoxicity of 5-FUdR, 5-FU and Tomudex in HeLa cells. *Br J Pharmacol.* 1999; 127(8): 1777-86.
31. Lin SB, Ts'o PO, Sun SK, et al. Inhibition of thymidylate synthase activity by antisense oligodeoxynucleotide and possible role in thymineless treatment. *Mol Pharmacol.* 2001; 60(3): 474-9.
32. Shimizu T, Nakagawa Y, Takahashi N, et al. Thymidylate synthase gene amplification predicts pemetrexed resistance in patients with advanced non-small cell lung cancer. *Clin Transl Oncol.* 2016; 18(1): 107-12.
33. Barclay J, Creswell J, León J. Cancer immunotherapy and the PD-1/PD-L1 checkpoint pathway. *Arch Esp Urol.* 2018; 71(4): 393-9.
34. Niemeijer AN, Leung D, Huisman MC, et al. Whole body PD-1 and PD-L1 positron emission tomography in patients with non-small-cell lung cancer. *Nat Commun.* 2018; 9(1): 4664.
35. Núñez F, Chipchase MD, Clarke AR, et al. Nucleotide excision repair gene (ERCC1) deficiency causes G(2) arrest in hepatocytes and a reduction in liver binucleation: the role of p53 and p21. *FASEB J.* 2000; 14(9): 1073-82.
36. Villalobos M, Czapiewski P, Reinmuth N, et al. ERCC1 Assessment in upfront treatment with and without cisplatin-based Chemotherapy in stage IIIB/IV non-squamous non-small Cell Lung cancer. *Med Oncol.* 2018; 35(7): 106.

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