Lung Cancer 122 (2018) 124–130

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Tumor-associated metabolic and inflammatory responses in early stage non-small cell lung cancer: Local patterns and prognostic significance

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ARTICLE INFO

Keywords:
IL1β
Pentose phosphate pathway
PDL1
Early-stage
NSCLC
Prognosis
Inflammation

ABSTRACT

Introduction: Non-small cell lung cancer (NSCLC) patients diagnosed in early stage and surgically-treated have five-year mortality rate > 20%. The identification of biomarkers able to predict progression and death may help to identify patients needing closer follow-up.

Methods: A retrospective cohort of early-stage surgically-treated NSCLC patients enrolled in the International Association for the Study of Lung Cancer (IASLC) Staging Project was created, and tissue Microarrays (TMAs) were constructed with tumor and non-tumor lung tissue. Pentose phosphate pathway (PPP) proteins (transketolase [TKT] and transketolase-like 1 [TKTL1]), inflammatory markers (cyclooxygenase-2 [COX-2], tumor necrosis factor alpha [TNF-α], interleukin 1 beta [IL1β], nuclear factor kappa-light-chain-enhancer of activated B cells [NFκB]-p65 and antigen Ki-67), and programmed death-ligand 1 (PDL1) were measured by immunohistochemistry.

Results: NSCLC patients with adenocarcinoma (ADC) or squamous cell carcinoma (SCC) were included in the study (n = 199). TKT and TKTL1 were significantly higher in ADC than in non-tumor tissue ($p < 0.001$). Higher values were also observed in NSCLC for all the inflammatory markers, with figures > 30% above those of non-tumor tissue ($p < 0.001$). PDL1 analysis showed a higher percentage of positivity in ADC than in non-tumor tissue ($p < 0.001$). Multivariate Cox proportional hazards modeling confirmed that high IL1β level in tumor tissue was independently associated with 3-year mortality in NSCLC [HR = 2.05, 95% CI (1.1–3.7), p = 0.019], a relationship driven by ADC subtype.

Conclusion: This study confirms an increase in metabolic activity and an inflammatory response in tumor tissue

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https://doi.org/10.1016/j.lungcan.2018.06.015
Received 16 January 2018; Received in revised form 27 April 2018; Accepted 9 June 2018

of early stage NSCLC, and a significant relationship between high levels of IL1β in the tumor and poor prognosis in ADC.

1. Introduction

Lung cancer (LC) is the leading cause of cancer-related mortality worldwide, accounting for 1.69 million out of the total of 8.8 million cancer-related deaths in 2015 [1]. Non-small cell lung carcinoma (NSCLC) is involved in up to 90% of LC cases, with adenocarcinoma (ADC) and squamous cell carcinoma (SCC) being the major subtypes [2]. Unfortunately, almost 85% of patients with LC remain undiagnosed until the disease is symptomatic and has reached an advanced stage [3], resulting in poor prognosis and an overall 5-year survival rate of less than 15% [4,5]. LC patients diagnosed in early stages and treated surgically have better prognosis, but their 5-year mortality is still above 20% [6]. Therefore, the identification of biomarkers able to predict which patients submitted to therapeutic surgery present a higher risk of progression and death in the following years may help to improve survival, through the introduction of adjuvant therapies and closer follow-up.

Carcinogenesis evolves through genetic and epigenetic changes which allow cells to acquire the specific characteristics of malignancy [7,8], but tumor progression also depends on complex interactions between host genetic susceptibility and the local environment [9]. The chronic and uncontrolled cell proliferation that characterizes carcinogenesis involves not only a deregulated control of cell proliferation, but also the adjustments of energy metabolism necessary to increase cell growth and division [8].The activity of the pentose phosphate pathway (PPP) enables tumor cell proliferation by generating pentose phosphates and ribonucleotides which favor the high rate of nucleic acid synthesis of cancer cells. This pathway is also a major source of nicotinamide adenide dinucleotide phosphate (NADPH), which is required for cell survival under stress conditions [10–12]. PPP contains two distinct metabolic branches, the oxidative and the non-oxidative branches. Flux through the oxidative branch is mainly regulated through enzymes such as glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate-dehydrogenase (6PGD). The enzyme transketolase (TKT) is one of the main regulators of the non-oxidative branch, while transketolase-like 1 (TKTL1) is an isoenzyme of TKT which is also thought to participate in the regulation of the PPP [13], although its precise role is still a matter of debate. TKTL1 has been shown to be upregulated in various cancer tissues, and its overexpression is correlated with many relevant cancer-related mechanisms such as invasiveness, therapeutic resistance, and poor prognosis [10,14,15].

The communication between tumor cells and their microenvironment (TME), which is composed of different cell subpopulations and an extracellular matrix (ECM), is also critical for tumor growth and progression [16,17]. Tumor stroma consists of fibroblasts, macrophage-lineage cells and vascular endothelial cells, with variable amounts of extracellular matrix, all of which contribute a support structure for tumor growth [18]. Inflammatory cells are a key component of the microenvironment of carcinomas and influence cancer initiation and promotion by secreting cytokines, growth factors and chemokines, which stimulate proliferation of epithelia as well as the generation of reactive oxygen species that can cause DNA damage [19]. Inflammation is involved in all stages of tumorigenesis, from malignant transformation and tumor initiation to the invasion and metastasis of established tumors [20].

Understanding the metabolic changes of tumor cells and the nature of their microenvironment is important for identifying prognostic markers in early stage LC, and may allow the development of LC therapies targeting aspects of TME which influence the disease's progression. The aim of the present study was to identify molecular biomarkers related to cell metabolism and local inflammation in cancer tissue from early stage surgically-treated NSCLC patients that may be a potential target for specific therapies, and its relationship to prognosis.

2. Methods

2.1. Design and population

The present study was nested in the International Association for the Study of Lung Cancer (IASLC) Staging Project, which has the aim of improving staging accuracy in LC. It was performed on the cases included by the Bronchogenic Carcinoma Cooperative Group of the Spanish Society of Pneumology and Thoracic Surgery (GCCB-II) in the Project between 2009 and 2012. The eighth edition of the tumor, node and metastasis (TNM) classification for LC was published in 2016 [21,22], and the innovations introduced in this edition were based on the data-driven recommendations of the IASLC [23,24]. The Project analyzed 77,154 evaluable patients recorded by 35 centers from 16 countries around the world [25]. Data entry and analysis were performed by Cancer Research and Biostatistics (CRAB), a non-profit organization based in Seattle, Washington. The inclusion criterion was a pathologic diagnosis of LC in patients without any associated severe renal or hepatic disease that might compromise survival in the following three years. The Spanish GCCB-II contributed 2362 prospectively registered cases to the IASLC International Database to inform the eighth edition of the TNM classification [26]. Eighteen of the hospitals participating in the GCCB-II included 1035 surgically resected LC patients, representing 42.8% of the Spanish cohort. In this study, we retrospectively analyzed tissue samples from these surgical patients enrolled in the IASLC Staging Project and recorded at the participating Spanish hospitals [27]. The research protocol was approved by the reference regional research and ethics committee for the study (Fundació Parc Taulí reference PI12/02040) and by the local research and ethics committees of all participating centers. Written informed consent was obtained from all participating patients in accordance with the current legal regulations (RD 1716/2011) in Spain.

2.2. Clinical variables

The baseline clinical variables included in the IASLC database have been described elsewhere [25,28]. In brief, baseline clinical information included demographic data, smoking status, comorbidities, tumor location, blood analyses, results from staging tests, lung function, details on surgical treatment, pathological diagnosis and TNM descriptors. Survival was assessed annually, and overall mortality three years after surgical treatment was considered the main outcome for the present study.

2.3. Sample processing

Formalin-fixed paraffin embedded tissue samples were obtained from participating hospitals and stored in the Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES) Pulmonary Biobank Platform (PBP), part of the Spanish Biobank Network [29]. A pathology panel formed by three experts evaluated the samples, confirmed the histology and selected the appropriate area to perform Tissue Microarrays (TMAs), identifying an area with abundant malignant cells and a second area with preserved lung tissue distant from the tumor when possible. TMAs were prepared at the Centro de Investigación Médica Aplicada (CIMA) of the Universidad de Navarra. From each block, three cylinders of 0.1 cm in diameter from a tumor zone and two cylinders from the non-tumor area were obtained.