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Original contribution

Specific pattern of LKB1 and phospho-acetyl-CoA carboxylase protein immunostaining in human normal tissues and lung carcinomas☆

Esther Conde MD, PhD[a,b], Ana Suarez-Gauthier MD[a], Elena García-García MD, PhD[b], Fernando Lopez-Rios MD, PhD[b], Angel Lopez-Encuentra MD, PhD[c], Ricardo García-Lujan MD, PhD[c], Manuel Morente MD, PhD[d], Lydia Sanchez-Verde PhD[e], Montserrat Sanchez-Cespedes PhD[a,*]

[a]*Lung Cancer Group, Pathology Programme, Spanish National Cancer Centre (CNIO), 28029 Madrid, Spain*
[b]*Pathology Department, Hospital Universitario 12 de Octubre, 28041 Madrid, Spain*
[c]*Pulmonary Department, Hospital Universitario 12 de Octubre, 28041 Madrid, Spain*
[d]*Tumour Bank, Spanish National Cancer Centre (CNIO), 28029 Madrid, Spain*
[e]*Immunohistochemistry-Histology Unit, Spanish National Cancer Centre (CNIO), 28029 Madrid, Spain*

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Summary The *LKB1* tumor suppressor gene codes for a serine/threonine protein kinase, and among its substrates is the adenosine monophosphate–dependent protein kinase, a sensor of intracellular energy levels. LKB1 is genetically inactivated in several types of tumors, especially lung adenocarcinomas. Here we used immunohistochemistry to evaluate the levels of LKB1 and the phosphorylated form of the acetyl-CoA carboxylase (ACC) protein in a variety of human adult normal tissues and in 159 lung carcinomas. The enzyme ACC becomes inactive upon phosphorylation by adenosine monophosphate–dependent protein kinase. Our analysis in normal tissues revealed strong LKB1 immunostaining in most epithelia, in the seminiferous tubules of the testis, in myocytes from skeletal muscle, and in glia cells. In contrast to the cytosolic location of LKB1 found in most tissues, glia cells carried mainly nuclear LKB1. Some epithelial cells showed apical accumulation of LKB1, supporting its role in cell polarity. Regarding phospho-ACC (p-ACC), strong immunostaining was observed in myocytes from the skeletal muscle and heart, and in Leydig cells of the testis. In lung tumors, LKB1 immunostaining was absent, moderate, and high in 20%, 61%, and 19% of the tumors, respectively, whereas p-ACC immunostaining was found to be absent/low, moderate, and high in 35%, 34%, and 31% of the tumors, respectively. High levels of LKB1 and p-ACC immunostaining predominated in lung adenocarcinomas compared with squamous cell carcinomas. Finally, high p-ACC was an independent marker for prediction of better survival in lung adenocarcinoma patients. Median overall survival was longer in patients with p-ACC–positive than those with p-ACC–negative

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* Corresponding author. Molecular Pathology Programme, Spanish National Cancer Centre (CNIO), 28029 Madrid, Spain.
E-mail address: msanchez@cnio.es (M. Sanchez-Cespedes).

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tumors (96 versus 44 months, $P = .04$). In conclusion, our observations provide complete information about the pattern and levels of LKB1 and p-ACC immunostaining in normal tissues and in lung tumors, and highlight the special relevance of abnormalities of the LKB1 pathway in lung adenocarcinoma.

1. Introduction

The human *LKB1* gene, also known as *STK11*, encodes a serine/threonine kinase of 433 amino acids that is involved in several cell functions, including cell polarity and signal transduction [1]. Germline mutations at *LKB1* cause Peutz-Jeghers syndrome (PJS), an autosomal dominantly inherited disorder that predisposes to multiple hamartomatous polyps in the gastrointestinal tract and mucocutaneous pigmented spots in different mucosae [2,3]. Compared with the general population, patients with PJS are highly predisposed to developing cancer in a variety of tissues, including colon, small intestine, breast, ovary, pancreas, and lung [4]. In addition to PJS, *LKB1* is also mutated in sporadic tumors, especially those of the lung [5,6]. At least one third of sporadic lung adenocarcinomas, but not other lung cancer histologies, carry *LKB1*-inactivating mutations. Closely related orthologues of *LKB1* have been found in mice, *Drosophila*, *Xenopus* (*Xeek1*), and *Caenorhabditis elegans* (*Par4*) [7-9]. *Lkb1* orthologues in *Xenopus* and *C. elegans* are involved in early embryonic development [8,10], and the *Lkb1* homologue in *Drosophila* is required for early anterior-posterior axis formation and epithelial polarity [9,11]. Moreover, it has recently been reported that LKB1 may participate in the regulation of the asymmetry of meiotic division and in the polarization of the oocyte during mouse oogenesis [12]. In humans, the LKB1 protein contains potential nuclear localization signals, between amino acids 38 and 43 [13], and it has been detected in both cytoplasm and nucleus [14-16].

Recent reports have revealed that LKB1 phosphorylates the adenosine monophosphate–activated protein kinase (AMPK) [17-19], a sensor of cellular energy status that becomes activated under several physiologic and pathologic stresses, including exercise, hypoxia, ischemia, heat shock, and low glucose. AMPK promotes ATP-generating pathways, including glucose transport, glycolysis, and fatty acid oxidation, while inhibiting energy-consuming anabolic pathways. Active AMPK phosphorylates multiple downstream targets, including acetyl-CoA carboxylase (ACC), the rate-limiting enzyme of the long-chain fatty acid synthesis that becomes inactive after phosphorylation at Ser-79 (phospho-ACC [p-ACC]). Therefore, detailed studies on the levels of LKB1 protein expression and of AMPK activation, evaluated through phosphorylation of its indirect target, ACC, in normal tissues should help us to understand the role of LKB1 in PJS and in lung carcinogenesis.

In the present work, we aimed to determine the profile of LKB1 and p-ACC protein immunostaining in a wide range of normal tissues and to understand the relevance and clinical impact of LKB1 and p-ACC abnormal immunostaining in lung tumors.

2. Materials and methods

2.1. Antibodies

Anti-LKB1 human monoclonal antibodies were developed in the Monoclonal Antibody Unit at the Spanish National Cancer Centre (CNIO). That used in the present study (Ley 37D/G6) is now commercially available from Abcam, Cambridge, UK and Santa Cruz Biotechnology (Santa Cruz, CA). Western blot analysis of LKB1 in lung cancer cells carrying LKB1-inactivating mutations or wild-type LKB1 demonstrated the complete absence or the presence of a unique band at 52 kd, respectively [20]. Anti–p-ACC (S79), which recognizes both α and β isoforms of ACC, was obtained from Cell Signaling Technology, Beverly, MA. Western blot analysis for anti–p-ACC protein is commonly used to asses the activity of AMPK and has been demonstrated to be specific for the recognition of p-ACC and not the total ACC protein [18,19].

2.2. Tissue selection

Anonymized tissue sections were obtained with informed patient consent and full local Medical Research Ethics Committee approval. Normal tissue sections were obtained from the Hospital Universitario 12 de Octubre, Madrid, Spain. The normal tissue examined included cerebral and cerebellar cortex, skin, salivary gland, esophagus, small and large intestine, stomach, pancreas, gallbladder, skeletal and smooth muscle, heart, blood vessel, tonsil, lymph node, thymus, thyroid, adrenal, kidney, urinary bladder, ovary, placenta, lung, prostate, breast, endometrial, cervix, ovarian, and testis. In addition, tumor samples were collected from 159 patients who underwent resection at the Hospital Universitario 12 de Octubre between 1993 and 1997. All tumors were classified by 3 pathologists (E. Conde, A. Suarez-Gauthier, and F. Lopez-Rios) according to the 2004 World Health Organization classification.

2.3. Tissue microarrays of normal and lung tumor tissues

A Tissue Arrayer (Beecher Instruments, Silver Spring, MD) was used to construct the tissue microarrays (TMAs)