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Original contribution

Expression of mTORC1/2-related proteins in primary and brain metastatic lung adenocarcinoma☆,☆☆,★

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Summary Brain metastases (BMs) are common complications of adenocarcinomas (ADCs) of the lung and are associated with a poor prognosis. Although an increasing amount of data indicates that dysregulated activity of mammalian target of rapamycin (mTOR) can influence the metastatic potential of various tumors, the role of mTOR complexes in the development of BMs from ADCs of the lung is largely unknown. To estimate mTOR activity, we studied the expression of mTOR-related proteins (mTORC1: p-mTOR, p-S6; mTORC2: p-mTOR, Rictor) in primary (n = 67) and brain metastatic (n = 67) lung ADCs, including 15 paired tissue samples, using immunohistochemistry and tissue microarrays. Correlation with clinicopathological parameters was also analyzed. Increased p-mTOR, p-S6, and Rictor expressions were observed in 34%, 33%, and 37% of primary ADCs and in 79%, 70%, and 66% of BMs, respectively. Expression of these markers was significantly higher in BMs as compared with primary carcinomas ($P < .0001$, $P < .0001$, $P < .001$). Rictor expression was significantly higher in primary ADCs of the paired cases with BMs as compared with primary ADCs without BMs (67% versus 28%; $P < .01$). No other statistically significant correlations were found between mTOR activity and clinicopathological parameters. The increased mTORC1/C2 activity in a subset of pulmonary ADCs and the higher incidence of increased mTORC1/

Abbreviations ADC, adenocarcinoma; AKT, protein kinase B; ALK, anaplastic lymphoma kinase; BM, brain metastasis; EGFR, epidermal growth factor receptor; IGFR-1, insulin-like growth factor receptor-1; IHC, immunohistochemistry; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; mTORC2, mTOR complex 2; NSCLC, non–small cell lung cancer; PDGFR, platelet-derived growth factor receptor; PI3K, phosphoinositide 3-kinase; PKC-α, protein kinase C-α; p-mTOR, phosphorylated mTOR; p-S6, phosphorylated ribosomal S6 kinase; Rictor, rapamycin-insensitive companion of mTOR; ROS-1, c-ros oncogene 1; TMA, tissue microarray; VEGFR, vascular endothelial growth factor; mTORI, mTOR inhibitor.

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C2 activity in BMs suggest that the immunohistochemistry panel for characterizing mTOR activity and its potential predictive and prognostic role warrants further investigations.

1. Introduction

Lung cancer, the leading cause of cancer death worldwide, accounts for approximately 40% to 50% of all brain metastases (BMs) [1]. BMs portend a poor prognosis despite the use of multimodal therapies [2,3]. Targetable genetic mutations in non–small cell lung cancer (NSCLC) currently include epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase, and c-ros oncogene 1 [4]. However, to further improve the clinical outcome of patients with brain metastatic NSCLC, additional targets are needed.

The phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway integrates several cell signals and regulates many normal cell functions. It is also one of the most frequently dysregulated signaling pathways in human tumors, including NSCLCs [5]. It can be activated by the EGFR, insulinlike growth factor receptor, vascular endothelial growth factor, and platelet-derived growth factor receptor membrane receptor families and various mutations in the *PI3KCA* gene [6,7]. Activation of the PI3K/AKT/mTOR pathway, in turn, may lead to tumor progression.

The serine/threonine kinase mTOR is an integral part of 2 different multiprotein complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). Raptor and Rictor are characteristic elements of mTORC1 and mTORC2, respectively. The most important targets of mTORC1 are the S6K and 4EBP-1 proteins. Their phosphorylation results in enhanced protein synthesis through S6 phosphorylation and the release of eukaryotic translation initiation factor 4E. In contrast, mTORC2 regulates the phosphorylation of AKT (Ser473), protein kinase C-α, paxillin and the small GTPases, RAC, and RHO. The 2 complexes differ not only in functions but also in their sensitivity to rapamycin; mTORC1 is sensitive and mTORC2 is considered to be resistant to the drug [8-10]. Long-term treatment with rapamycin, however, can also disrupt mTORC2 assembly and activity [11].

Animal studies have demonstrated that everolimus, temsirolimus, and other next-generation mTOR inhibitors (mTORIs) penetrate the blood-brain barrier [12,13]. In addition, these mTORIs have also shown promise in treating gliomas and breast carcinomas metastatic to the brain [14,15].

To identify potential predictive biomarkers that could guide targeted therapy by mTORIs, we analyzed the expression of mTORC1- and mTORC2-related proteins in primary adenocarcinomas (ADCs) of the lung and brain metastatic ADCs of lung origin.

2. Materials and methods

2.1. Patients and tissue samples

Primary (n = 67) and brain metastatic (n = 67) pulmonary ADCs, including a subset with matched primaries and BMs (n = 15), were studied. The tumors were obtained by surgical resection at the National Korányi Institute of TB and Pulmonology (Budapest, Hungary), the Bajcsy-Zsilinszky Hospital (Budapest), and the National Institute of Clinical Neurosciences (Budapest) between January 2003 and December 2011. The archived tissue samples were used with the approval of the Hungarian Scientific Council National Ethics Committee for Scientific Research (No. 510/2013, 86/2015).

The tumors were re-reviewed and reclassified according to the 2015 *World Health Organization Classification of Lung Tumours* [16]. Clinicopathological data, such as age, sex, stage of primary ADC at diagnosis (as per the 2015 *World Health Organization Classification of Lung Tumours*), smoking status, size, and multiplicity of BMs were obtained from medical records.

Table 1 Summary of antibodies and their conditions used for IHC in this study

Antibody	Clone	Manufacturer	Dilution	Antigen retrieval (buffer)	Detection system
Anti–phospho-mTOR	#2976	Cell Signaling (Danvers, MA)	1:100	Pressure cooker (CA)	Novolink (Novocastra, Wetzlar, Germany)
Anti–phospho-S6	#2211	Cell Signaling	1:100	Pressure cooker (CA)	Novolink
Anti-Rictor	A500-002A	Bethyl Laboratories (Montgomery, TX)	1:1000	Pressure cooker (CA)	Vectastain Elite Universal (Vector Laboratories, Burlingame, CA)

Abbreviation: CA, citric acid buffer (pH 6.0).