ORIGINAL ARTICLE

PI3K as a Potential Therapeutic Target in Thymic Epithelial Tumors

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ABSTRACT

Introduction: Thymic epithelial tumors (TETs) are rare tumors originating from the epithelium of the thymus with limited therapeutic options beyond surgery. The pathogenesis of TETs is poorly understood, and the scarcity of model systems for these rare tumors makes the study of their biology very challenging.

Methods: A new cell line (MP57) was established from a thymic carcinoma specimen and characterized using standard biomarker analysis, as well as next-generation sequencing (NGS) and functional assays. Sanger sequencing was used to confirm the mutations identified by NGS.

Results: MP57 possesses all the tested thymic epithelial markers and is deemed a bona fide thymic carcinoma cell line. NGS analysis of MP57 identified a mutation in the gene *PIK3R2*, which encodes a regulatory subunit of PI3K. Further analysis identified different mutations in multiple *PI3K* subunit genes in another cell line and several primary thymic carcinoma samples, including two catalytic subunits (*PIK3CA* and *PIK3CG*) and another regulatory subunit (*PIK3R4*). Inhibiting PI3K with GDC-0941 resulted in in vitro antitumor activity in TET cells carrying mutant PI3K subunits.

Conclusions: Alterations of PI3K due to mutations in its catalytic or regulatory subunits are observed in a subgroup of TETs, in particular, thymic carcinomas. Targeting PI3K may be an effective strategy to treat these tumors.

Keywords: Thymic epithelial tumor; PI3K; Mutation; PI3K inhibitor

Introduction

Thymic epithelial tumors (TETs) are rare tumors originating from epithelial cells of the thymus with an annual incidence of 0.13 per 100,000 persons in the United States. On the basis of the World Health Organization (WHO) classification system, TETs are histologically classified into thymoma types A, AB, B1, B2, and B3 and thymic carcinomas.[1] Thymomas maintain the architectural structure of the thymus, whereas thymic carcinomas are not recognizable from carcinomas that arise in other sites. The WHO histological classification has prognostic value,[2,3] and thymoma types A, AB, and B1 have a 10-year survival rate of more than 80%.[4] Thymomas B2 and B3 have an intermediate behavior, and thymic carcinomas are aggressive tumors: they exhibit cytological atypia and high histological heterogeneity, lack typical structure of the thymus, and have higher metastatic potential, with 5-year survival rates at approximately 50%.[5,6] Surgical resection is the main therapeutic intervention for TETs at early stages, whereas advanced and recurrent disease are treated with chemotherapy.[7–13]

Although targeted therapies have produced successful results in other cancer types in selected patient

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populations, only a few clinical trials have shown some efficacy with targeted treatments in TETs.[2,11,14,15] This is largely due to lack of understanding of the biology of these tumors. However, recent genetic studies using comparative genomic hybridization, whole genome expression analysis, and next-generation sequencing (NGS) have provided new insights into the biology of this rare cancer. Thymic carcinomas have a distinct molecular signature that segregates them from the thymoma subtypes.[6,16–23] Such genetic differences, combined with the histology-based classification system, are clinically relevant for both prognosis and treatment of TETs and may contribute to identification of new molecular targets for therapeutic intervention.[3,7,19,20]

The rarity of TETs represents a great challenge to study of the biology of these tumors as well as to the development of novel therapeutic strategies. The challenge is in large part due to the shortage of model systems, and currently there are only six cell lines available for this tumor type.[24–28] Here, we report the establishment of a new cell line (designated MP57) derived from a patient with thymic carcinoma. Through characterization of MP57 along with other cell lines and primary tumors, we identified five actionable mutations in four different subunits of PI3K. These mutations were mostly found in thymic carcinomas. We further demonstrated that inhibiting PI3K has in vitro antitumor activity in the TET cells carrying mutant PI3K subunits.

Materials and Methods

Cell Lines

MP57 is a thymic carcinoma cell line newly established from a tumor specimen collected at autopsy. The patient's history has been described elsewhere.[9] Tumor cells were retrieved through autopsy from the primary mediastinal mass. The solid tumor was dissociated into a single-cell suspension by magnetic-activated cell sorting (MACS) using a gentleMACS dissociator (Miltenyi Biotech, San Diego, CA). After dissociation, the sample was cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Life Technologies, Grand Island, NY) in an incubator with a 5% CO_2 atmosphere at 37°C. The medium was replaced every 3 or 4 days. T1889 is a thymic carcinoma cell line kindly provided by Dr. Marco Breinig,[29] and IU-TAB1 is a type AB thymoma cell line kindly provided by Dr. G. W. Sledge Jr.[27] All these cell lines are cultured in RPMI plus 10% fetal bovine serum and 1% penicillin/streptomycin.

IF Staining

MP57 cells were seeded on 12-mm cover glass coated with Poly-L-Lysine (Corning Inc., Corning, NY). Sixteen hours later, cells were fixed in 4% formaldehyde for 15 minutes and incubated with blocking buffer (1× phosphate-buffered saline [PBS], pH 7, plus 8% bovine serum albumin and 0.3% Triton X-100) for 60 minutes. Cells were then incubated for 1 hour at room temperature with different primary antibodies, followed by incubation with the secondary antibody AlexaFluor488 and 4,6-diamino-2-phenylindole (DAPI) for 90 minutes. After immunofluorescence (IF) staining, the cover glasses were mounted with coverslips and photos were taken on a fluorescence microscope. Primary antibodies for epithelial cell adhesion molecule (EpCAM), E-cadherin, c-KIT, p63, vimentin, and N-cadherin were purchased from Cell Signaling Technologies (Danvers, MA). Anti-cytokeratin clone AE1/AE3 antibody was obtained from Dako (Carpinteria, CA), and the AlexaFluor 488 and DAPI were purchased from Invitrogen/Life Technologies.

Flow Cytometric Analysis

Cells were trypsinized, washed in 1× PBS (pH 7.4), centrifuged at 350× g for 5 minutes, and resuspended in ice-cold staining buffer (1× PBS, pH 7.4, plus 1% bovine serum albumin). Cells were then incubated with the fluorochrome-conjugated antibodies for 20 minutes on ice, and flow cytometric analysis of the stained cells was performed using a FACSCalibur cell sorter (BD Biosciences, San Jose, CA). APC anti-human c-Kit (clone 104D2), Phycoerythrin (PE) anti-human EpCAM (clone 9CD), and PE/Cy7 anti-human E-cadherin antibodies were purchased from BioLegend (San Diego, CA). Anti-fibroblast-PE (human, clone: REA165) was obtained by MACS (Miltenyi Biotec).

Cell Proliferation Assay

Cells were seeded at a density of 5000 cells per well in 96-well plates for the CellTiter-Glo Luminescent Cell Viability Assay (Promega, Madison, WI). The plates were incubated for 24, 48, or 72 hours at 37°C in 5% CO_2 before 100 μL Cell Titer-Glo reagent was added to lyse the cells. After an incubation period of 10 minutes at room temperature, the luminescence intensity was measured using the GloMax Multidetection system (Promega).

The trypan blue dye exclusion assay was used as an alternative method to assess cell proliferation. Cells (5×10^4 cells per well) were seeded in 24-well plates. After 24, 48, and 72 hours, cells were detached from the well using TrypLe (Life Technologies, Carlsbad, CA) and resuspended in medium. Cell viability was evaluated by adding trypan blue solution (0.4% in PBS) (Life Technologies) to the cell suspension. After 3 minutes of incubation, the unstained (nonviable) and stained (viable) cells were counted in a hemacytometer.