Production and Characterization of an Antibody Specific for a Novel Protein Serine/Threonine Kinase, MPK38, Highly Expressed in Hematopoietic Cells

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Abstract

We report an antibody that selectively recognizes MPK38, a new protein serine/threonine kinase closely related to the SNF1 serine/threonine kinase family. This antibody recognized a region of the N-terminal kinase catalytic domain and part of the remaining C-terminal portion and was sensitive enough to detect a 72-kDa recombinant MPK38 in insect cells by Western blotting. Immunoblot analysis showed that the recombinant MPK38 was expressed in a time-dependent manner and reached a maximum after 48 h postinfection. In addition, the immune complex kinase assay revealed that the recombinant and endogenous MPK38 protein autophosphorylated in vitro. Phosphoamino acid analysis of autophosphorylated MPK38 protein showed that the phosphorylation was exclusively on serine and threonine residues, suggesting that MPK38 is a protein serine/threonine kinase. Thus, this antibody could be helpful for elucidating the biological functions of MPK38 in the MPK38-expressing cells.

Index Entries: Anti-MPK38 antibody; serine/threonine kinase; autophosphorylation; signal transduction; recombinant protein.

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Introduction

Protein phosphorylation plays a cardinal role in many signal transduction pathways. In higher eukaryotes, the regulation of protein phosphorylation is important in cellular events such as proliferation, oncogenesis, differentiation, and development (1). The importance of protein kinases in regulating phosphorylation is underscored by the large number of protein kinase genes present in eukaryotes (2). Recently nearly 2000 conventional protein kinases have been estimated. All protein kinases have been classified into two broad subfamilies based on substrate specificity: serine/threonine specific and tyrosine specific (3).

We previously reported the cloning of MPK38 gene, a novel protein serine/threonine kinase, from a cDNA library constructed from the murine teratocarcinoma PCC4 cell line (4). MPK38 was also recently cloned and given the name Melk for maternal embryonic leucine zipper kinase by Heyer et al. (5). MPK38 is a putative member of the serine/threonine kinase family and shows extensive amino acid sequence homology with the SNF1 serine/threonine kinase family. In budding yeast, the derepression of the glucose-repressible genes is required for the function of a complex signaling when the yeast is deprived of glucose in the environment. One of these, SNF1, encodes a protein serine/threonine kinase and plays a major role in regulating glucose-repressed genes in response to glucose limitation (6). Recently, several SNF1-related kinases have been identified from both mammals and plants and have been shown to complement functionally the sucrose nonfermenting 1 (snf1) mutants that are unable to utilize sucrose, raffinose, galactose, maltose, and other sugars as a carbon source (7–11). Su et al. (12) recently reported that a novel serine/threonine kinase, called XEEK1, expressed in Xenopus was most similar to the SNF1 kinase with about 65% similarity within the catalytic domain, and that it could not functionally complement on snf1 mutation in yeast, suggesting that XEEK1 is not functionally related to the yeast SNF1 kinase or its related kinases. Therefore, it has been unclear whether or not the MPK38 kinase exhibiting approx 60% protein sequence identity can complement the yeast snf1 mutation.

In the present study, to provide a system for functional analysis of MPK38, we have expressed a truncated version encoding the catalytic kinase domain of MPK38 as a T7-Tag-tagged recombinant protein in bacteria and generated a specific antibody against the N-terminal catalytic domain of MPK38. In addition, we describe the biochemical characterizations of the MPK38 kinase.

Materials and Methods

Cell Culture and Reagents

Murine hematopoietic lineage cell lines (R1.1 and EL4, T lymphomas; A20, B lymphoma) were maintained in RPMI-1640 medium supplemented