Mol Cell Biochem (2009) 328:201–208
DOI 10.1007/s11010-009-0090-9

MAP kinases in proliferating human colon cancer Caco-2 cells

Natalia Buzzi · Andrea Colicheo · Ricardo Boland · Ana Russo de Boland

Received: 30 September 2008 / Accepted: 11 March 2009 / Published online: 20 March 2009

Abstract The mitogen-activated protein kinase (MAPK) cascade is one of the most ubiquitous signal transduction systems and is rapidly activated by various stimuli, such as cellular stress and death. The Caco-2 cell line is an in vitro model for colon cancer studies. We investigated the activation status of the ERK1/2, p38, JNK1/2, and ERK5 kinases and their respective upstream intracellular activators in Caco-2 cells induced to proliferate by 10% fetal bovine serum (FBS). The states of phosphorylation of the above MAPKs and their upstream kinases, MEK1/2, MKK3/6, MKK4, and MKK7, respectively, were studied by Western blot analysis. Phosphorylation was barely detectable before serum stimulation, and the stimulation of cell proliferation by the addition of FBS increased MEK1/2 and ERK1/2 phosphorylation 2 to 3 fold after 3 min. FBS stimulated p38 and MKK3/6 to the same extent within 2 min of treatment and JNK1/2 and its upstream kinases MKK4 and MKK7 5-fold (3 min). Addition of FBS also rapidly phosphorylated ERK5 (2 to 3.5-fold between 2 and 5 min) and the transcription factor CREB. Incubation of Caco-2 cells with FBS was followed by a rapid induction of c-Fos and c-Myc expression. Studies with ERK1/2 specific inhibitor PD98059, p38 MAPK inhibitor SB203580, or JNK inhibitor SP600125 showed that FBS regulates Caco-2 cell proliferation via the three MAPK pathways.

Keywords Caco-2 cells · FBS · ERK · JNK · p38 MAPK

N. Buzzi · A. Colicheo · R. Boland · A. R. de Boland (✉)
Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, San Juan 670, 8000 Bahía Blanca, Argentina
e-mail: aboland@criba.edu.ar

Introduction

Mitogen-activated protein kinases (MAPKs) are ubiquitous enzymes involved in signal transduction [1]. Their activity is essential in numerous cellular functions, including proliferation and programmed cell death.

Mammalian MAPKs are commonly divided into subfamilies that include the Extra-cellular regulated protein kinases (ERKs), the c-jun N-terminal kinases (JNKs), the p38, and the Big MAP kinases (BMKs), but more subfamilies may exist [2]. All MAPKs are activated via a unique dual phosphorylation mechanism, on a Thr-X-Tyr motif, located in the phosphorylation loop. Dual specificity kinases, termed MAP kinase kinases (MAPKKs, MEKs, or MKKs), catalyze this dual phosphorylation. MAPKKs are not highly specific, and may phosphorylate all members of a subfamily or even of two families [2]. Extracellular signal-regulated kinases (ERK 1 and 2) are the best-characterized MAPKs [3]. The p38 MAPKs are strongly activated under stress conditions, and therefore, together with the JNKs, are also known as Stress Activated Protein Kinases (SAPKs).

The substrate selectivity of MAPKs is conferred by specific interaction motifs located on the kinases and substrates [4, 5]. In addition, MAPK cascade specificity is also mediated by scaffolding proteins that organize pathways in specific modules through simultaneous binding of several components [6]. Activated MAPKs further propagate the signal by phosphorylating downstream targets such as transcription factors and other kinases.

A number of research groups have turned to cell lines derived from human colonic adenocarcinomas [7], several of which retain features of the differentiated intestinal epithelial cell. One such cell line, known as Caco-2, is human colonic adenocarcinoma cells that have been