Human Pathology (2013) 44, 766–776

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Elk-1 phosphorylated at threonine-417 is present in diverse cancers and correlates with differentiation grade of colonic adenocarcinoma☆

Jacqueline F. Morris BS[a], Jai-Yoon Sul PhD[a], Min-Sun Kim MD, PhD[b], Andres J. Klein-Szanto MD, PhD[c], Terri Schochet PhD[a], Anil Rustgi MD[d], James H. Eberwine PhD[a,*]

[a]*Department of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104, USA*
[b]*Department of Physiology, Wonkwang University School of Medicine, Iksan 570-749, South Korea*
[c]*Department of Pathology, Fox Chase Cancer Center, Philadelphia, PA 19111, USA*
[d]*Division of Gastroenterology, Department of Medicine and Genetics, Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA 19104, USA*

Received 22 March 2012; revised 3 August 2012; accepted 3 August 2012

Keywords:
Adenocarcinoma;
Tumor grade;
Elk-1;
Phosphorylation;
Colorectal cancer

Summary Elk-1 is a member of the Ets family of transcription factors, which are identified by a conserved Ets DNA-binding domain that mediates transcriptional regulation at Ets sequence–containing promoters. The activation domain of Elk-1 is important for executing its physiologic functions and contains many phosphorylation sites targeted by various MAP kinases following exposure to cell stressors or mitogenic stimuli. The different combinations of phosphorylated sites allow specificity of cellular responses mediated through redundant signaling pathways activated by distinct stimuli. Through phosphorylation of S383, mitogen-activated protein kinase (MAPK)–activating stimuli have been shown to regulate various processes important in carcinogenesis through transcriptional regulation in various cell lines, including proliferation. Phosphorylation at the T417 site (pT417), but not the S383 site, is involved in neuronal apoptosis induced through dendritic signaling mechanisms and associates with neuronal lesions in many Lewy body diseases. This points to distinct roles for these different phosphorylation sites in pathophysiologic pathways. However, the S383 site remains the best characterized in the context of normal function and carcinogenesis in cell lines, and less is known about the biochemistry of other phosphorylation sites, particularly in more biochemically relevant models. Here, we show that Elk-1 pT417 is present in epithelial cell nuclei of various normal and cancer tissues and that the number of pT417-positive cells correlates with differentiation grade of colonic adenocarcinomas. This nuclear localization and correlation with tumor differentiation in adenocarcinoma suggests a potentially important transcriptional and biochemical role of this phosphorylation site in carcinogenesis of this tumor type.

1. Introduction

Elk-1 is a member of the Ets oncogene family of transcription factors, which are characterized by a conserved DNA-binding domain, or Ets domain. Phosphorylation of Elk-

☆ Funding sources: This work was funded by NIH grant MH078136.
* Corresponding author. Department of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104, USA.
E-mail address: eberwine@upenn.edu (J. H. Eberwine).

0046-8177/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.humpath.2012.08.001

1 at S383 serves as an integration point within cells for redundant activation of upstream mitogen-activated protein kinase (MAPK) signaling cascades by distinct external stimuli. Growth factors and mitogens activate the Raf/mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) pathway, resulting in regulation of growth and differentiation, through transcriptional activation by Elk-1 pS383 [1,2]. Stress, inflammatory cytokines, and other growth factors activate the p38 MAPK and stress-activated protein kinase (SAPK)/Jun N-terminal kinase (JNK) pathways, resulting in regulation of inflammation, apoptosis, growth, and differentiation, also through transcriptional activation by Elk-1 at pS383 [1,2]. Elk-1 regulates transcription by interacting with serum response factor at serum response elements within the promoters of genes that subsequently execute the functions initiated by extracellular signals [3]. Elk-1 is most notably known for contributing to regulation of growth and proliferation through transcriptional activation of immediate early genes such as *c-fos* and *ZIF-268* [4,5].

The transcriptional activation of Elk-1 through phosphorylation at S383 has been extensively studied in many different cell lines. Yet, the fact that these cellular processes respond to different extracellular signaling molecules through common MAPK pathways that all culminate in activation of Elk-1 suggests the need for different phosphorylation patterns of Elk-1 to execute different cellular functions. For example, induction of ternary complex formation of Elk-1 with serum response factor following nerve growth factor (NGF) stimulation was shown to be dependent on phosphorylation at S383 and S389 by ERK1/2 in PC-12 cells [6]. Complex formation was enhanced by phosphorylation at S324 or S422 but inhibited by phosphorylation at T336 [6]. No phosphorylation was detected at T353, T363, T368, or T417 [6]. Mutation of S383 or S389 or both T363 and S422 or both T417 and S422 greatly reduced transcriptional activation of Elk-1 in response to epidermal growth factor (EGF) in NIH 3T3 fibroblasts [7]. 12-O-tetradecanoylphorbol-13-acetate (TPA) increased Elk-1 transcriptional activity in COS cells through phosphorylation of Elk-1 by ERK1/2 at S324, T336, S383, S389, or S422 [8]. Thus, although ERK1/2 was involved in phosphorylation of Elk-1 under all conditions, differentiation between stimuli was made possible through different phosphorylation patterns of Elk-1.

Transcriptional activation of Elk-1 through these signaling cascades results in regulation of a wide range of normal cellular functions, including cell-cell and cell-matrix adhesion, proliferation, and apoptosis [9,10]. Carcinogenesis involves dysregulation of these processes, and Elk-1 activation has been implicated in cancers of many tissues including breast, pancreas, and colon. Inhibition of Elk-1 in the breast cancer cell line MCF-7 enhanced the antiproliferative effects of breast cancer 1a/1b (BRCA1a/1b) in a MEK/ERK pathway–dependent manner, presumably through interaction of its Ets DNA binding domain and subsequent inhibition of *c-fos* transcription [11]. Using the breast cancer cell lines MCF-7 and SK-BR-3, it was shown that EGF activation of human epidermal growth factor receptor 2 (HER-2) stimulated Elk-1 phosphorylation at S383 through the MEK/ERK pathway and promoted cell survival [12]. Knockdown of Elk-1 in an oncogenic RAS–expressing human pancreatic cancer cell line resulted in inhibition of proliferation of the cancer cells [13]. In the rat small intestine–derived RIE-1 cell line, Elk-1 phosphorylation at S383 by ERK1/2 resulted in increased transcription of cycloocygenase-2 (COX-2), which caused loss of cell-matrix adhesion and decreased apoptosis [14].

Despite its nuclear site of action as a transcription factor, Elk-1 protein and messenger RNA (mRNA) have been found in the processes of cultured hippocampal neurons [4,15], and this localization was found to impact pathophysiologic responses. Elk-1 mRNA introduced specifically into neuronal dendrites, but not the soma, using a laser-based, site-directed transfection procedure known as phototransfection, initiated apoptosis [16]. This response was shown to be dependent on local translation of Elk-1 within the dendrite and on transcription [16]. In addition, this response was shown to be dependent on the phosphorylation state of Elk-1 [17]. The activation domain of Elk-1 contains many phosphorylation sites, which, excluding the S383 site, remain largely uncharacterized [6]. Mutation of these sites to create nonphosphorylatable mutants revealed that preventing phosphorylation at the T417 site, but not the S383 site, blocked the apoptotic response when the mutant mRNA were introduced into neuronal dendrites [17]. Phosphorylation at the T417 site was later shown to be an important marker for Lewy bodies in Parkinson disease, whereas other phosphoforms were not [17]. These studies suggest a role for this transcription factor outside the nucleus as well as providing impetus for characterizing the physiologic effects of other phosphorylation sites within the activation domain of Elk-1, particularly the T417 site.

Much of what is known about Elk-1 function in carcinogenic processes has been obtained from cell lines, which, although useful for approximating biochemical responses in cancer tissue, have a limited capacity to do so. This is especially important in colorectal cancer and medulloblastoma where sonic hedgehog signaling is important in promoting in vivo carcinogenesis [18,19], but signaling components are down-regulated in cultured tumor cells [19,20], limiting their utility in biochemical studies. In addition, Elk-1 activation in these studies is measured by phosphorylation of Elk-1 at S383, with no evaluation of the contribution of other phosphorylation sites.

Given these limitations in cell lines, the role of Elk-1 in many carcinogenic processes, its phosphosite specificity in pathophysiology, and the limited amount of information on the role of the T417 site in pathophysiology, the staining patterns for Elk-1 phosphorylated at T417 in various cancers and normal tissues was determined to identify its potential contribution to carcinogenesis. Elk-1 pT417 was found in many normal and cancer tissues screened in a tissue microarray. A screen of adenocarcinomas of the colon revealed higher Elk-1 pT417 staining in tumors compared