bHLH-zip Transcription Factor Spz1 Mediates Mitogen-Activated Protein Kinase Cell Proliferation, Transformation, and Tumorigenesis

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Abstract

bHLH-zip proteins usually play important regulatory roles in cell growth and differentiation. In this study, we show that Spz1, a bHLH-zip transcription factor, acts downstream of mitogen-activated protein kinase (MAPK, extracellular signal-regulated kinase 1/2) to up-regulate cell proliferation and tumorigenesis. In addition, through an interaction with proliferating cell nuclear antigen (PCNA) promoter, Spz1 induced cell proliferation concomitant with an increase in PCNA gene expression. Spz1-transfected cells formed colony foci on soft agar and developed fibrosarcoma tumors in nude mice. MAPK directly interacted and phosphorylated Spz1 protein, which increased PCNA transcription and cell tumorigenic activities. Reduction of endogenous Spz1 expression via RNA interference decreased cell proliferation in p19 embryonic carcinoma cells. High levels of Spz1 expression were detected in murine tumor cell lines and tumor samples of both human and Spz1 transgenic mice. Thus, Spz1 may act as a proto-oncogene, participating in the MAPK signal pathway, and be a potential therapeutic target in the treatment of Ras-induced tumors. (Cancer Res 2005; 65(10): 4041-50)

Introduction

Cancer directly affects at least one third of the human population (1, 2). Despite this extensive research prevalence, the genetic determinants of cancer risk remain largely unknown. Highly potent mutations of oncogenes as well as tumor suppressor genes, and more subtle and complex genetic interactions within each individual are currently thought to influence susceptibility to cancers (1, 3–5).

The proto-oncogene Ras plays an important role both in cell growth and in the regulation of differentiation (2, 5, 6). The Ras gene product (Ras) mediates cellular responses to several mitogens, growth factors, and external stimuli (7, 8). These stimuli in turn activate a series of signal transduction pathways (5, 6, 8). The best-known Ras regulated pathway is mediated via Raf, a serine/threonine kinase. The Ras-Raf interaction leads to a conformational change in Raf that unmask one or two phosphorylation residues and stabilizes a new catalytic domain (9, 10). The stabilized protein subsequently phosphorylates and regulates other kinases, designated MAPKK (MEK1/2) and mitogen-activated protein kinase (MAPK, extracellular signal-regulated kinase 1/2). This activity transduces the Ras signals into cell nuclei (11). However, although many factors are regulated through Ras signaling pathways in cells, the factors directly involved in Ras-induced tumor progression are still unknown.

bHLH-zip proteins are one of the transcriptional regulator families involved in the control of cell growth and differentiation (12, 13). Inappropriate expression of bHLH-zip genes (e.g., c-myc) is frequently associated with tumorigenesis and abnormal development. Alternations in the level or structure of c-Myc protein are the root cause of many malignancies in human and other animals (14, 15). Transgenic mice expressing c-Myc in hematopoietic cells have malignant lymphoid and myeloid tumors (16). In this study, we show that Spz1, a bHLH-zip protein, acts as a proto-oncogene regulated by a MAPK signaling pathway that can increase cell proliferation and tumorigenic activity as well as leading to primary tumor formation in nude and transgenic mice.

Materials and Methods

Plasmids. Proliferating cell nuclear antigen (PCNA) promoter region (1,268 bp) was amplified with primer 1, 5′-GAATTCTGCTGACCAAGGTA-3′ and primer 2, 5′-CTAGGCCGTCAAGCCTGAATTA-3′ (17) and subcloned into pGL2-basic luciferase reporter vector (Promega, Madison, WI). A pSV40-BLC vector (Promega) was used to normalize the transfection efficiency of different expression vectors. Ras and Raf dominant-negative constructs were kindly provided by Dr. H.M. Shih (Division of Molecular and Genomic Medicine, National Health Research Institute, Taipei, Taiwan; ref. 18).

The RNA interference plasmid was constructed by subcloning a 57-nt double-strand oligonucleotide, from which a Spz1 24-nucleotide sequence was separated from the reverse complement sequence by a short spacer, using a Q-TOF Ultima MALDI instrument (Micromass, Manchester, United Kingdom).

Thymidine incorporation. Spz1-transfected cells (2 × 10^4) were seeded in 96-well culture plates in DMEM, containing 0.1% fetal bovine serum (FBS) and incubated at 37°C for 24 hours. After washing with PBS, the medium was replaced by DMEM with a normal serum concentration (10%) and 5 μCi [3H]-thymidine and incubated at 37°C for another 12 hours. Cells were harvested and transferred to glass fiber filters (Packard, Chicago, IL). Thymidine incorporation was quantitated with MATRIX 96 (Packard).

Western blot and immunohistochemical staining analysis. Western blot analysis and immunohistochemical (fluorescence) staining were done as previously described (19, 20). The primary antibodies used in this study were actin polyclonal antibody (1:2,000, Santa Cruz Biotechnology, Santa

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Cruz, CA), PCNA polyclonal antibody (1:2,000, Santa Cruz Biotechnology), phospho-ERK1/2 antibody (1:2,000, Cell Signaling Technology, Beverly, MA), Flag-tag (1:200, Upstate, Lake Placid, NY), FITC-conjugated anti-mouse, alkaline phosphatase–conjugated anti-rabbit antibody (1:500, Jackson ImmunoResearch Laboratories, West Grove, PA), and Spz1 rabbit polyclonal antibody (1:5,000; refs. 19, 20). B23 primary antibody was provided by Dr. Benjamin Y.M. Yung (Graduate Institute of Pharmacology, National Yang Ming University, Taipei, Republic of China; ref. 21).

Chromatin immunoprecipitation assays. The chromatin immunoprecipitation (ChIP) assays were analyzed with a ChIP kit according to the manufacturer’s instructions (Upstate). Chromatin immunoprecipitation assays were analyzed with a ChIP kit according to the manufacturer’s instructions. Different protein kinase inhibitors (U0126, 5 and 25 μmol/L) were added to the culture medium 24 hours after transfection and relative luciferase activities were measured according to the manufacturer’s instructions. Different protein kinase inhibitors (U0126, 5 and 25 μmol/L) were added to the culture medium 24 hours after transfection.

Immunoprecipitation and protein kinase assays. Immunoprecipitation assays were done as previously described (19). Anti-Flag antibody and anti–phospho-ERK1/2 were used to immunoprecipitate protein. The resuspended IP complexes were analyzed by Western blot. Purified MEX1/2, ERK1/2 (Upstate), glutathione S-transferase (GST)–tagged recombinant P38 kinase, and c-jun NH2-terminal kinase (JNK, Calbiochem, La Jolla, CA) were used for in vitro protein kinase assays. All reactions were done according to the manufacturer’s instructions. Inactivated ERK1, MBP, and c-Jun–GST were used as positive control substrates for different activated protein kinases.

Anchorage-independent growth assays. Cells (104 or 5 × 105) in 1-mL culture medium were mixed with an equal volume of 0.6% of top agar and plated onto 60-mm dishes with 0.5% bottom agar. Plates were incubated at 37°C for 2 weeks. Colonies were visualized by staining with 0.05% crystal violet acetate and colonies larger than 0.5 mm were counted. Different protein kinase inhibitors (U0126, 5 and 25 μmol/L) were added to the culture medium 24 hours after transfection.

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Results

Ectopic Spz1 expression changes cellular morphology and cell cycle distribution and increases cell proliferation. To elucidate the cellular functions of Spz1, epithelial (Cos-7) and fibroblast (HEL 299) cell lines with stably expressed wild-type or mutant Flag-SPz1 constructs, M1 (335→A and 355→L) and M2 (1065→L) were established and characterized. Spz1 expression (green) was detected predominantly in cell nuclei by confocal microscopy (Fig. 1A). Ectopic expression of Spz1 resulted in cells showing relatively longitudinal fibroblast morphology. Cytoskeletal distribution (shown by filamentous actin, F-actin, staining) in Spz1-transfected cells was more organized than in vector-transfected cells. The morphology of M1-transfected cells was similar to that of wild-type Spz1-transfected cells but with some mutated Spz1 proteins diffused in the cytoplasm. However, the M2 mutant was mostly detected in the cytoplasm and the morphology of transfected cells was looser and more irregular than that in spz1- and M1-transfected cells (Fig. 1A). Growth rate comparisons between Spz1- and vector-transfected cells revealed ectopic Spz1 expression to be associated with a duplication time 4 to 8 hours shorter than that of vector-transfected cells (Fig. 1B, left). The rate of cell proliferation, as determined by [3H]-thymidine incorporation, showed a 5-fold increase in Spz1-transfected Cos-7 cells and a 2-fold increase in Spz1-transfected HEL 299 cells compared with vector-transfected cells (Fig. 1B, right).

The effects of Spz1 on cell cycle progression were analyzed by flow cytometry. As shown in Fig. 1C, the G2-M- and S-phase cell populations in Spz1-transfected cells were about 8% and 5% greater than those in vector-transfected cells. This result was confirmed by bromodeoxyuridine (BrdU) incorporation assays. As shown in Fig. 1D, there were 18% more BrdU-incorporated cells in the Spz1-expressing cell population than in vector-transfected cells. These results implied an overexpression of Spz1-accelerated cell cycle progression with a shorter G1 phase. Even under starvation for 24 hours, Spz1-expressing cells still had a higher G2-M population than vector-transfected cells, despite a slight increase in apoptotic cell number (sub-G1; Fig. 1C).

Spz1 up-regulates proliferating cell nuclear antigen expression. To identify gene expression influenced by Spz1 overexpression, HEL 299 and Cos-7 cell lysates were prepared after transient transfection and analyzed with two-dimensional electrophoresis. Differentially expressed proteins were analyzed with matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. One of those identified was the PCNA (the cell proliferation marker). As shown in Fig. 2A, PCNA expression increased 3.3-fold in Spz1-transfected cells compared with vector-transfected cells. To investigate whether PCNA is a direct downstream target of Spz1, constructs with a luciferase reporter gene driven by series deletions of the PCNA promoter fragment were cotransfected with the Spz1 expression construct into Cos-7 cells. As shown in Fig. 2B, after subtraction of the endogenous activity with vector-only expression construct, Spz1 expression induced the activity of PCNA promoter by 3- to 4-fold. The transactivity of Spz1 dropped to a basal level in cells transfected with only the upstream 46 bp of the PCNA promoter.

Spz1 directly binds to proliferating cell nuclear antigen promoter. The interaction of Spz1 with consensus guanine-rich (GGG/AGGG/AA/TT) and E-box motifs identified from our previous study (19) further suggest that Spz1 might regulate PCNA expression through direct binding to its promoter. Three guanine-rich containing segments −178 to −202 bp (E1), −144 to −168 bp (E2), and −106 to −130 bp (E3) were identified in the PCNA promoter (Fig. 2C). By electrophoresis mobility shift assays, the strongest Spz1 binding affinity was identified in the E1 region (Fig. 2C). The luciferase activities decreased significantly in the reporter assay with E1 deleted from the −233-bp promoter construct but not in constructs containing deletions of the E2 or E3 motifs (Fig. 2C).

A ChiP assay was used to determine whether Spz1 binds the PCNA promoter in vivo. The PCNA promoter was precipitated using an anti-Spz1 antibody from HEL 299 cells cultured in normal media (10% FBS) or treated with SB203580 (P38 kinase inhibitor) but at a much reduced level under conditions of starvation (0.5% FBS) or MEK inhibitor, U0126-treated medium (Fig. 2D). Mutant Spz1 protein in M2-transfected cells showed a much weaker
Figure 1. Cell morphology and proliferation rate altered with ectopic Spz1 expression. 
A, Spz1 localization and cytoskeletal remodeling in Spz1-transfected Cos-7 cells. (Spz1, green; cytoskeleton (F-actin), red; nucleus, blue; magnification, 400×) B, cell growth curves and [\(^{3}H\)]-thymidine incorporation rate of Spz1- and vector-transfected cells (n = 6). C, cell cycle progression of Spz1-expressing cells identified by flow cytometry. D, DNA synthesis rate of vector- and Spz1-transfected cells analyzed by BrdU incorporation assay (arrow, BrdU-labeled cells; n = 3) *, P < 0.05; **, P < 0.01.
binding activity for PCNA promoter than the wild-type or M1 mutant Spz1 proteins (Fig. 2D). These observations indicate that the up-regulation of PCNA by Spz1 overexpression was through direct binding of Spz1 to the PCNA promoter E1 region and that MEK1/2 signaling regulated this interaction.

Mitogen-activated protein kinase (ERK1/2) interacts and phosphorylates Spz1 directly. The enhancement of cell proliferation and altered cytoskeleton remodeling seen in Spz1 expressing cell lines (Cos-7 and HEL 299) were similar to the biological responses induced by Ras in several cell lines (5, 6, 22). To elucidate whether Spz1 transactivation of PCNA is influenced by the Ras signaling pathway, both dominant-negative mutants of Ras or Raf were individually cotransfected with Spz1 expression constructs into Cos-7 cells. PCNA promoter-driven luciferase activities induced by Spz1 was abolished by deletion of the PCNA promoter. Spz1 binding affinity to guanine-rich regions on the PCNA promoter was determined by electrophoresis mobility shift assays (left). N, probe only; B, BSA only; and C, immunoprecipitate of Cos-7 cells with normal goat serum. The increased luciferase activity of the –233 bp PCNA promoter induced by Spz1 was abolished by deletion of the PCNA E1 region (right).

As shown in Fig. 3B, ectopic and endogenous Spz1 were present in both the cytoplasm and nuclear extract. In addition,
phospho-ERK1/2 was strongly detected in Spz1 immunoprecipitates and Spz1 protein was also detected in phospho-ERK1/2 immunoprecipitates (Fig. 3C). In vitro kinase assays showed that activated ERK1/2 could phosphorylate Spz1, M1, and M2 proteins (Fig. 3D, top); however, no phosphorylation of Spz1 was detected after the addition of p38 kinase and JNK. This in vitro Spz1 phosphorylation by ERK1/2 was confirmed under normal culture conditions (Fig. 3D, bottom). The phosphorylation level was abolished by MEK inhibitor, U0126, and starvation medium (0.5% FBS) but not by SB203580, p38 kinase inhibitor. The mutations on the three putative phosphorylation sites did not effect the ERK1/2 phosphorylation because the M1 and M2 mutants exhibited a

Figure 3. Transactivity of Spz1 is MEK1/2 dependent. A, dominant-negative Ras, Ras-DN (Raf-DN) and MEK inhibitors (U0126) down-regulated Spz1-induced PCNA promoter activity dose-dependently (n = 3). B, Spz1 protein is expressed in both cytoplasm (C) and nucleus (N). B23, nuclear extract indicator. C, Spz1 interacts directly with phospho-ERK1/2. Phospho-ERK1/2 was detected in Spz1 immunoprecipitate in p19 cells (top) and Spz1 was also detected in ERK1/2 immunoprecipitate (bottom). C, with pCl-neo vector only (n = 3). D, in vitro and in vivo protein kinase assays. Activated ERK1/2 directly phosphorylates Spz1, M1, and M2 protein in vitro and this phosphorylation activity was abolished when cells were treated with MEK inhibitor (U0126) but not with p38 kinase inhibitor, SB203580. *** P < 0.005.
phosphorylation level similar to wild-type Spz1 (Fig. 3D). These results showed that activated ERK1/2 can directly bind to and phosphorylate Spz1 in vitro and in vivo.

**Ectopic Spz1 expression induces tumorigenic activity in fibroblast and epithelial cells.** To study Spz1 tumorigenic activity, constructs containing wild-type Spz1, M1, and M2 were stably transfected into both fibroblast (HEL 299) and epithelial cells (Cos-7 and TM4), which were subjected to anchorage-independent growth assays. These cell foci were counted as shown in Fig. 4A. The transformed foci from the wild-type and M1 mutant in Cos-7 and TM4 cells appeared 2 weeks after cells were seeded onto soft agar, whereas the vector- and M2-transfected cells showed almost no foci formation. In HEL 299 cells, foci formed 4 weeks after seeding (Fig. 4A, left). These results imply that ectopic Spz1 expression could transform cells and that residue 106S in Spz1 is critical for this transforming activity. These cellular transforming tests were done with MEK inhibitor (U0126) and p38 kinase inhibitor (SB203580). As shown in Fig. 4A (right), U0126 significantly reduced the transformation activities induced by wild-type Spz1 in Cos-7 cells in a dose-dependent manner. These results further confirm that ectopic Spz1 expression can induce cell tumorigenesis in vitro and this transforming activity is regulated by the MEK/ERK signaling pathway.

**Tumorigenic potential of Spz1-transfected cell.** The tumorigenic ability of Spz1 in vivo was further examined by s.c. injection of Spz1-expressing cells into nude mice. Five to 8 weeks post-injection, all mice displayed primary tumors at the Spz1 cell injection site, whereas no tumors were detected at the sites that

Figure 4. Ectopic Spz1-induced cellular tumorigenic activity. A, in vitro transformation activity of Spz1. The transformation activities of Spz-transfected Cos-7 and HEL 299 cells were determined by anchorage-independent growth assay and transformed foci numbers were quantitated (left). MEK1/2 inhibitors reduced the number of transformed foci induced by Spz1 (right; n = 4), *, P < 0.05; **, P < 0.01. B, ectopic Spz1-induced tumor formation in nude mice. Physical appearance of a representative mouse was observed 6 weeks after injection with $10^6$ cells. C, tumor growth curves in nude mice after injection of different cells (n = 10). D, histologic and immunohistochemical analysis of muscular fibrosarcoma induced by ectopic Spz1 expression in nude mice. Arrows, intersecting fascicles formed from a well-differentiated fibrosarcoma.
received vector-transfected cells (Fig. 4B). The tumors swiftly increased in size 5 weeks after injection of wild-type and M1-transfected Cos-7 cells. However, M2-transfected cells only induced a tiny tumor, even 6 weeks after injection (Fig. 4C, top).

Furthermore, Spz1-transfected HEL 299 fibroblast and TM4 epithelial cells displayed similar tumorigenic potential as Cos-7 epithelial cells, except that tumor formation was slower (Fig. 4C, bottom).

The solid and well-defined s.c. fibrosacoma tumors formed in nude mice from exposure to Spz1 overexpressing Cos-7 and HEL 299 cells were dissected and analyzed. H&E staining of tumor sections showed some uniform intersecting fascicles (fascicular pattern) consisting of spindle cells with only slight variation in size and shape (Fig. 4D). High levels of Spz1 expression were immunohistochemically confirmed in these tumor cells (Fig. 4D).

Spz1 expression required in cell proliferation and tumor progression. To elucidate if Spz1 expression was critical for mouse tumor formation, a construct constitutively expressing Spz1 short hairpin RNA was transfected into Cos-7 (with ectopic Spz1) and p19 (with endogenous Spz1) cells. As shown in Fig. 5A, the short hairpin construct knocked down Spz1 expression by about 70% to 80%. Compared with wild-type p19 cells, Spz1 RNA interfering cells showed a differentiated morphology with an irregular cell shape and widespread silk-like connections between cells (Fig. 5B). The proliferation rate of Spz1 knockdown cells was decreased by 70% in comparison with vector-transfected cells as determined by [3H]-thymidine incorporation (Fig. 5C). The tumorigenic activity of Spz1 knockdown cells was also significantly reduced, as shown by the decreased tumor volume developed in nude mice (Fig. 5D).

Ectopic Spz1 expression leads to tumor progression in transgenic mice. Transgenic mice ectopically expressing Spz1 under the control of cytomegalovirus promoter have been established recently (20). We found that 15% to 20% of those transgenic mice formed primary tumors in the lung, liver, kidney, small intestine, and uterine tissues individually at 6 to 8 months of age (Fig. 6A). In primary lung tumors, the pulmonary blastoma showed a regular size and morphology, and it contained epithelial and mesenchymal cells in firm mass (arrow). The secondary carcinoma, lymphoplasmacytic lymphoma, showed an extensive permeation in sinunoids by pleomorphic undifferentiated,
basophilic hyperchromatic nuclei (arrow). In the kidney, the primary tumor cells, such as human Wilms’ tumor, showed deeply stained basophilic nuclei (arrow) and several mitotic figures. Higher levels of Spz1 expression were also detected in these tumor regions compared with the nontumor regions (Fig. 6A). These results suggest that high level of ectopic Spz1 expression correlates with tumorigenicity in vivo.

**Ectopic Spz1 expression detected in mouse tumor cell lines.** Mouse tumor cell lines and human brain cancer samples were further examined to elucidate whether Spz1 expression also correlated with murine tumors and human tumors. For this, Spz1 expression in several murine tumor cell lines was examined by Northern Blot analysis. Among the 12 cell lines characterized, four disparate tumor cell lines, RAW264.7 (macrophage-like tumor), M-MSV-BALB3T3 (embryo sarcoma), p19 (teratocarcinoma), and R1.1 (lymphoma), were identified as exhibiting high levels of Spz1 expression (Fig. 6B). Some glioma and astroma from human brain cancer samples also showed high levels of Spz1 expression (Fig. 6B). This data further implies the tumorigenic activity of Spz1 in murine and human tissues.

**Figure 6.** Ectopic Spz1-induced tumorigenicity in transgenic mice and mouse tumor cell lines. A, different types of primary tumors identified in 6- to 8-month-old Spz1 transgenic mice. The high level of Spz1 expression in uterine tumor section was shown by immunohistochemical staining analysis. Small blocks at the right corners are the enlarged histogram (400×) of each panel. B, Spz1 expression in multiple mouse tumor cell lines (left) and human brain tumor tissues (right). G, glioma; m, standard marker; N, normal; R, radionecrosis; A, astroma; LM, liver spine meta.; M, meningioma; O, oligodendroglialoma.
Discussion

In the present study, we have identified the bHLH-zip transcription factor Spz1, as a candidate proto-oncogene. Analysis of the Ras signaling pathway in vitro and in vivo revealed that activated MAPK (ERK1/2) directly phosphorylates Spz1 and further stimulates tumorigenic activity in mice. Fibrosarcomas and different primary tumors were also observed in nude mice and in Spz1 transgenic mice.

From the cells transfected with Spz1 expression construct, we identified that PCNA is a downstream target of Spz1. PCNA is a highly conserved nuclear antigen protein and is implicated in cell cycle control (23–25), tumor progression (26–28), DNA replication (29–31), and DNA repair (32, 33). Overexpression in rapidly proliferating tumor cells has made PCNA a useful index for cell proliferation in tumor progression (26, 27, 34, 35). Our previous studies showed that Spz1 could bind to the E-box or to a conserved guanine-rich GGG/AGGGG/A DNA motif (19). In this study, we further show that Spz1 directly binds to a guanine-rich region of PCNA promoter and up-regulates its expression, which in turn induces cell transformation and mouse tumor formation. Studies using dominant-negative constructs and antagonists further revealed that the upstream regulatory system of the Spz1-PCNA cascade is the Raf/MEK/ERK signal pathway. Spz1 might be a novel Raf/MEK/ERK downstream target protein through a direct binding to ERK1/2, which executes Ras-like cellular transforming activities resulting in tumor formation. Spz1 could thus be a novel therapeutic target for Ras-induced cancers.

Phosphorylation and nuclear localization of ERK1/2 downstream targets is critical for execution of the function of Raf/MEK signaling (22, 36). In this study, nuclear localization of Spz1 has also been proved to be essential for its tumorigenicity. The fact that mutation of Spz1’s putative phosphorylation site abrogated its tumorigenicity suggests that the transforming activity of Spz1 may involve its nuclear localization. Although the phosphorylation level of M2 protein showed no significant difference from that of wild-type or M1 Spz1 proteins, phosphorylation that occurred at sites other than site 106 of M2 Spz1 protein could lead to interference so the reduced phosphorylation at site 106 could not be observed. Furthermore, the M2 mutation site is close to the ERK1/2 docking domain (D domain; 115-127 amino acids) and this mutation might influence the binding of MAPK kinase (36). In most cases, nuclear transport and export are regulated by phosphorylation, as shown for the yeast transcription factor Pho4 (38) and the mammalian factor NF-AT (39). Several signal transducers, including MAP and abl kinases, regulatory molecules of transcription such as Janus-activated kinase/signal transducers and activators of transcription, Smads, and JAB1, have been shown to translocate from the cytoplasm to nucleus and thereby transmit signals into the nucleus (40, 41). Some evidence suggests that improper subcellular localization of oncoproteins or tumor suppressors is related to tumor formation (36, 42). Recently, Spz1 was suggested to be a specific binding partner of the γ2 catalytic subunit of protein phosphatase-1 (43). This interaction between Spz1 and PPIcy2 was shown to prevent binding of the latter to a specific DNA sequence, the consensus E-box promoter sequence (43). Our results confirmed that the cellular function of Spz1 is correlated to its phosphorylation and cellular distribution.

In conclusion, endogenous and ectopic Spz1 can increase cell proliferation and cellular tumorigenic activity via the regulation of PCNA gene expression. Spz1 ectopic expression also seems essential for tumor formation and progression in the embryo carcinoma cell line p19. From this study, we suggest that Spz1 may be a proto-oncogene and play an important role in the Ras/Raf tumorigenic pathway.

Acknowledgments

Received 10/12/2004; revised 2/4/2005; accepted 2/9/2005.

Grant support: National Science Council, Taiwan grant NSC-92-2320-B-001-052.

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We thank D. K. Deen for his critical reading of this article.

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