Journal of Cellular Biochemistry 59:143–150 (1995)

ARTICLES

Association of Protein Kinase F_A/GSK-3α (a Proline-Directed Kinase and a Regulator of Protooncogenes) With Human Cervical Carcinoma Dedifferentiation/Progression

Shiaw-Der Yang, Jau-Song Yu, Tsong-Tze Lee, Mei-Hui Ni, Chuan-Ching Yang, Yat-Sen Ho, and Tze-Zen Tsen

Department of Cell and Molecular Biology, Institute of Basic Medicine, Chang Gung Medical College, Tao-Yuan (S.-D.Y., J.-S.Y., M.-H.N., C.-C.Y.), Institute of Biomedical and Life Sciences, National Tsing Hua University, Hsinchu (S.-D.Y., T.-T.L.), and Department of Pathology (Y.-S.H.) and Gynaecology and Obstetrics (T.-Z.T.), Chang Gung Memorial Hospital, Tao-Yuan, Taiwan, Republic of China

Abstract Computer analysis of protein phosphorylation-sites sequence revealed that most transcriptional factors and viral oncoproteins are prime targets for regulation of proline-directed protein phosphorylation, suggesting an association of proline-directed protein kinase (PDPK) family with neoplastic transformation and tumorigenesis. In this report, an immunoprecipitate activity assay of protein kinase F_A/glycogen synthase kinase-3α (kinase F_A/GSK-3α) (a particular member of PDPK family) has been optimized for human cervical tissue and used to demonstrate for the first time significantly increased ($P < 0.001$) activity in poorly differentiated cervical carcinoma (82.8 ± 6.6 U/mg of protein), moderately differentiated carcinoma (36.2 ± 3.4 U/mg of protein), and well-differentiated carcinoma (18.3 ± 2.4 U/mg of protein) from 36 human cervical carcinoma samples when compared to 12 normal controls (4.9 ± 0.6 U/mg of protein). Immunoblotting analysis further revealed that increased activity of kinase F_A/GSK-3α in cervical carcinoma is due to overexpression of protein synthesis of the kinase. Taken together, the results provide initial evidence that overexpression of protein synthesis and cellular activity of kinase F_A/GSK-3α may be involved in human cervical carcinoma dedifferentiation/progression, supporting an association of proline-directed protein kinase with neoplastic transformation and tumorigenesis. Since protein kinase F_A/GSK-3α may function as a possible regulator of transcription factors/proto-oncogenes, the results further suggest that kinase F_A/GSK-3α may play a potential role in human cervical carcinogenesis, especially in its dedifferentiation and progression.

Key words: cervical carcinoma progression, kinase F_A/GSK-3α, overexpression

Protein kinase F_A was originally identified as an activating factor of Mg.ATP-dependent protein phosphatase [Yang et al., 1980; Vandenheede et al., 1980] but has subsequently been identified as a protein kinase identical to glycogen synthase kinase-3α (GSK-3α) [Hemmings et al., 1981; Woodgett, 1990]. In addition to Mg.ATP-dependent protein phosphatase and glycogen synthase as its substrates, protein kinase F_A/GSK-3α was further identified as a multisubstrate protein kinase that could act on many substrates including the R_{II} subunit of cAMP-dependent protein kinase [Hemmings et al., 1982], phosphatase inhibitor-2 [Depaoli-Roach, 1984; Jurgensen et al., 1984], myelin basic protein [Yang, 1986], the nerve growth factor receptor [Taniuchi et al., 1986], the G subunit of phosphatase-1 [Fiol et al., 1988; Dent et al., 1989], neural cell adhesion molecule [Mackie et al., 1989], ATP-citrate lyase [Ramakrishna et al., 1990], neurofilament proteins [Guan et al., 1991], acetyl-CoA carboxylase [Hughes et al., 1992], microtubule associated protein-2 and tau protein [Yang et al., 1991, 1993; Hanger et al., 1992; Mandelkow et al., 1992], and brain cla-

Abbreviations used: GSK-3α, glycogen synthase kinase-3α; kinase F_A, type-1 protein phosphatase activating factor; MBP, myelin basic protein; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis.

Received January 12, 1995; accepted February 7, 1995.

Address reprint requests to Dr. S.-D. Yang, Department of Cell and Molecular Biology, Institute of Basic Medicine, Chang Gung Medical College, Tao-Yuan, Taiwan, ROC.

thrin-coated vesicles [Yu and Yang, 1993a]. Due to its unique feature as a multisubstrate protein kinase and as an activating factor of a multisubstrate protein phophatase, kinase F_A/GSK-3α may simultaneously modulate phosphorylation and dephosphorylation states of many key regulatory proteins involved in the regulation of diverse cell functions [Yang, 1991; Woodgett, 1991; Plyte et al., 1992]. Recently, kinase F_A/GSK-3α has further been identified as a possible regulator of the transcription factors/proto-oncogenes, such as c-jun [Boyle et al., 1991; de Groot et al., 1992], c-myb and c-myc [Plyte et al., 1992], CREM [de Groot et al., 1993], and CREB [Wang et al., 1994]. This raised another possibility that kinase F_A/GSK-3α could be involved in wider aspects of cellular regulation such as in control of nuclear transcription and tumor promotion. On the other hand, kinase F_A/GSK-3α has been demonstrated as a member of the so-called proline-directed protein kinase family [Hemmings and Cohen, 1983; Vulliet et al., 1989; Ramakrishna et al., 1990; Yang et al., 1993; Yu and Yang, 1994a]. Based on computer-assisted sequence analysis of transcriptional factors and viral oncoproteins, proline-directed protein phospharylation sites appeared to be a major regulatory mechanism [Suzuki, 1989] and proline-directed protein kinases such as kinase F_A/GSK-3α may therefore be possibly associated with neoplastic transformation and tumorigenesis. In a recent report [Lee et al., 1995], we identified that overexpression of kinase F_A/GSK-3α correlates with the state of dedifferentiation in human thyroid cells from normal, hyperplasia, adenoma to carcinoma stage, suggesting an association of kinase F_A/GSK-3α with human thyroid tumor cell dedifferentiation. However, since there is no sufficient data to support that thyroid cancer represents a progression from hyperplasia to adenoma to carcinoma, an association of kinase F_A/GSK-3α with human cancer progression therefore remains to be further established.

In this report, we tried to use human cervical carcinoma at well-, moderately, and poorly differentiated stages as a model system to test a possible involvement of kinase F_A/GSK-3α in human cancer progression and demonstrated that the cellular activity and protein level of kinase F_A/GSK-3α in invasive squamous cell carcinoma appeared to be many-fold higher than those of normal controls, and the expression of F_A/GSK-3α activity was significantly correlated with the degree of dedifferentiation/progression of cervical carcinoma, providing initial evidence that dysregulation and overexpression of kinase F_A/GSK-3α (a proline-directed protein kinase and a possible regulator of transcription factors/proto-oncogenes) is associated with human cervical carcinoma dedifferentiation/progression.

EXPERIMENTAL PROCEDURES

Materials

[γ-^{32}P]ATP was purchased from Amersham (Buckinghamshire, UK). Polyvinylidene fluoride (PVDF) membrane (Immobilon-P) was from Millipore (Bedford, MA). Sodium orthovanadate, Tween 20, and goat antirabbit IgG antibody conjugated with alkaline phosphatase were from Sigma (St. Louis, MO). Molecular weight marker proteins was from Boehringer Mannheim (Mannheim, Germany). BCA protein assay reagent was from Pierce (Rockford, IL). Alkaline phosphatase conjugate substrate kit was from Bio-Rad (Hercules, CA). Protein A-Sepharose CL-4B was from Pharmacia (Uppsala, Sweden).

Protein Purification

Myelin basic protein (MBP) was purified from porcine brain following the procedure as described in previous reports [Yang et al., 1987; Yu and Yang, 1994a].

Production of Anti-Kinase F_A/GSK-3α Antibody

The anti-kinase F_A/GSK-3α antibody was produced by using the peptide, TETQTGQDWQAPDA, corresponding to the carboxyl-terminal regions from amino acids 462–475 of the sequence of kinase F_A/GSK-3α [Woodgett, 1990] as the antigen. Production, affinity-purification, identification, and characterization of anti-kinase F_A/GSK-3α antibody were detailed in previous reports [Yu and Yang, 1993b, 1994b,c]. In this report, the antibody can potently and specifically immunoblot kinase F_A/GSK-3α from the human cervical tissue extracts on SDS-PAGE. The antibody can also efficiently immunoprecipitate all the kinase F_A/GSK-3α from the cervical tissue extracts and without blocking the kinase activity essentially as described in previous reports [Yu and Yang, 1994b,c] (data not shown).

Tissue Preparation

Cervical specimens were obtained during operation at the inpatient clinic of the Chang Gung

Memorial Hospital. The tissues were partly fixed in 10% formalin and embedded in paraffin for pathologic study and partly quick-frozen in liquid nitrogen for biochemical and immunological study. Light microsopy revealed that 12 sections were normal and 36 had invasive carcinomas of the cervix. Using the World Health Organization Histologic Classification of Invasive Squamous Cell Carcinoma, the 36 invasive cervical carcinomas were further classified as follows: 12 Grade I, highly differentiated carcinoma containing abundant keratin, keratin pearls, clearly visible intercellular bridges, and fewer than two mitoic figures per high-power microscopic field; 12 Grade II, moderately differentiated carcinoma containing less apparent keratin and two to four mitotic figures per high-power field and anisokaryosis; 12 Grade III, poorly differentiated carcinoma with no obvious keratin and more than four mitotic figures per high-power field and considerable pleomorphism of cells.

Frozen cervical tissues were homogenized in 3.5 volumes of solution A (20 mM Tris-HCl at pH 7.0, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 1 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride, 1 mM TLCK, 50 mM NaF, 0.2 mM sodium orthovanadate) on ice by a 5-ml Teflon pestle-fitted glass homogenizer (Wheaton, Millville, NJ) with 10 up-and-down strokes. After centrifugation at 400*g* for 1 min at 4°C to remove cell debris, the resulting cloudy supernatants were further sonicated on ice by Sonicator (model W-380, Heat Systems-Ultrasonics, Farmingdale, NY) for 3 × 10 s at 50% power output and then centrifuged at 20,000*g* for 20 min at 4°C. The resulting supernatants were used as the cervical tissue extracts.

Immunoprecipitation and Kinase F_A/GSK-3α Activity Assays in the Immunoprecipitates

Before immunoprecipitation, protein concentrations of the tissue extracts were first diluted to equal amounts with solution A. For immunoprecipitation, 200 μl of tissue extracts (~1 mg/ml protein) was incubated with 2 μl of affinity-purified anti-kinase F_A/GSK-3α antibody (10 mg/ml pure IgG) at 4°C for 1 h and then with 100 μl of protein A-Sepharose CL-4B (20% v/v, in solution A) for another 1 h with shaking. The immunoprecipitates were collected by centrifugation, washed three times with 1 ml of 0.5 M NaCl, once with 1 ml solution B (20 mM Tris-HCl at pH 7.0, 0.5 mM dithiothreitol, 1 mM phenylmethyl-sulfonyl fluoride, 1 mM benzamidine, 0.5 μg/ml aprotinin), and resuspended in 100 μl of solution B. For kinase F_A/GSK-3α activity assay in the immunoprecipitate, 15 μl of immunoprecipitate prepared as described above was incubated with 30 μl of a mixture containing 20 mM Tris-HCl at pH 7.0, 0.5 mM dithiothreitol, 0.2 mM [γ-^{32}P]ATP, 20 mM $MgCl_2$, and 4 mg/ml MBP at 30°C for 10 min. ^{32}P incorporation into MBP was measured by spotting 30 μl of reaction mixture on phosphocellulose paper (1 × 2 cm) (Whatman, Maidstone, UK), washing three times with 75 mM H_3PO_4, and counting in liquid scintillation analyzer (Model 1600CA, Packard, Meriden, CT) essentially as described in previous reports [Yang, 1986; Yu and Yang, 1994b,c]. A unit of kinase F_A/GSK-3α is that amount of enzyme that incorporates 1 pmol of phosphate/min into the MBP substrate.

Immunoblots

Proteins were transferred from unstained SDS-gels to Immobilon-P membrane in a Transphor (Hoefer, San Francisco, CA) at 350 mA in transfer buffer (10 mM 3-[cyclohexylamino]-1-propane-sulfonic acid [CAPS] at pH 10, and 20% methanol) at 4°C for 2 h. The membrane was incubated in TTBS buffer (20 mM Tris-HCl at pH 7.4, 0.5 M NaCl and 0.05% Tween 20) containing 5% non-fat dried milk at room temperature for 1 h to block the free protein binding sites. After washing 3 times with TTBS buffer, the membrane was incubated with 1 μg/ml anti-kinase F_A/GSK-3α antibody in TTBS buffer containing 3% non-fat dried milk at room temperature for 4 h, washed 3 times in TTBS buffer, and then incubated with secondary goat anti-rabbit IgG antibody conjugated with alkaline phosphatase diluted at 1/2,000 in TTBS buffer containing 3% non-fat dried milk at room temperature for 40 min and washed 3 times in TTBS buffer. Finally, the kinase F_A/GSK-3α protein was detected by the color development reagent kit.

Analytic Methods

Protein concentrations were determined by using the BCA protein assay reagent from Pierce. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method of Laemmli [1970] using 10% gels. Molecular weight markers used are as follows: α_2-macroglobulin (170,000), β-galatosidase (116,400), fructose-6-phosphate kinase (85,200), glutamate dehydrogenase (55,600), aldolase (39,200), and triose phosphate isomerase (26,600). Quan-

tification of the relative amounts of kinase F_A/GSK-3α on immunoblot was performed by densitometric scanning using a Video Densitometer (Biomed Instruments, Fullerton, CA).

Statistical Analysis

Results are means ± SD for n observations. Student's *t*-test was used to calculate the statistical significance of the differences.

RESULTS

Figure 1 depicts the biological activities of protein kinase F_A/GSK-3α in the immunoprecipitates obtained from the cervical tissue supernatant fluids of three human cervical carcinoma samples with different differentiation stages and of one normal control using a specific and potent anti-kinase F_A/GSK-3α antibody produced and affinity-purified as described in Experimental Procedures. From this figure it can be seen that the cellular activity of kinase F_A/GSK-3α appeared to be significantly increased in cervical carcinoma when compared to normal control. More interestingly, the cellular activity of kinase F_A/GSK-3α appeared to be proportionally increased following dedifferentiation/progression of human cervical carcinoma (Fig. 1). This is the first indication for an association of kinase F_A/GSK-3α with human cervical carcinoma dedifferentiation and progression. To confirm this point, we further analyzed 36 human cervical carcinoma at well-, moderately, and poorly differentiated stages and 12 normal controls. As shown in Table I, the cellular activity of kinase F_A/GSK-3α appeared to be statistically and significantly increased in poorly differentiated cervical carcinoma (82.8 ± 6.6 U/mg of protein), moderately differentiated carcinoma (36.2 ± 3.4 U/mg of protein), and well-differentiated carcinoma (18.3 ± 2.4 U/mg of protein) when compared to normal controls (4.9 ± 0.6 U/mg of protein). Most importantly, the cellular activity of kinase F_A/GSK-3α was significantly correlated with the degree of dedifferentiation of human cervical carcinoma and appeared to be statistically and proportionally increased following the progression of human cervical carcinoma (Table I), supporting an association of kinase F_A/GSK-3α with human cervical carcinoma dedifferentiation and progression.

Immunoblotting analysis of the tissue extracts (Fig. 2a) from three typical cervical carcinoma at different differentiation stages and one normal control further revealed that the increased cellular activity of kinase F_A/GSK-3α in cervical carcinoma is due to overexpression of protein synthesis of the kinase. Most importantly, the overexpressed protein levels of kinase F_A/GSK-3α, which are in agreement with the overexpressed cellular activities of kinase F_A/GSK-3α as shown in Figure 1 and Table I, also appeared to be significantly and proportionally correlated with the degree of dedifferentiation and progression of the cervical carcinoma (Fig. 2a). Quantification of kinase F_A/GSK-3α on the immunoblot by densitometric analysis (Fig. 2b) further revealed that the increased protein level for kinase F_A/GSK-3α is correlated with an increase in biological activity of kinase F_A/GSK-3α following the human cervical carcinoma dedifferentiation/progression when both expressed as percentage of controls (see Figs. 1 and 2). The same observations could also be extended to the 36 cervical carcinoma samples and 12 normal controls as described in Table I and Figures 1 and 2. All the results taken together demonstrate that overexpression of protein levels and cellular activities of protein kinase F_A/GSK-3α is indeed associated with

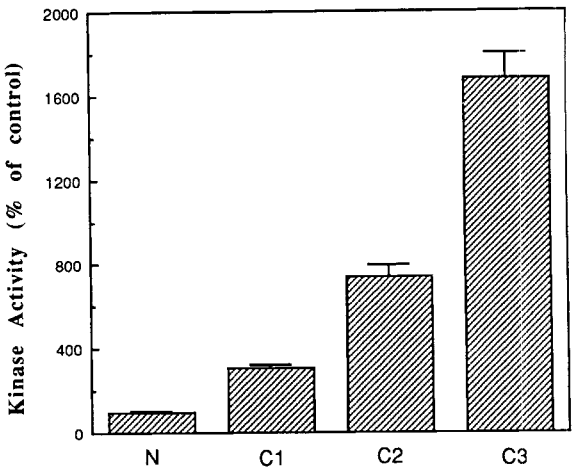

Fig. 1. Cellular activity of kinase F_A/GSK-3α immunoprecipitated from crude extracts of cervical tissues from normal control and different malignant lesions of the cervix. Crude extracts (~1 mg/ml of tissue protein in 200 μl of solution A) of cervical tissues from normal control and different malignant lesions of the cervix were immunoprecipitated by 20 μg anti-kinase F_A/GSK-3α antibody, followed by kinase activity assay in the immunoprecipitates as described under Experimental Procedures. *Lane N*, normal control; *lanes C1–C3*, cervical carcinoma at well-, moderately, and poorly differentiated stages, respectively. Data were taken from the averages of three independent experiments using one single tissue and expressed as means ± SD.

TABLE I. Protein Kinase F_A/GSK-3α Activity in Normal Cervix and Different Malignant Lesions of the Cervix*

Cervical tissues	Kinase F_A/GSK-3α activity in immunoprecipitate (U/mg)
Normal control (N = 12)	4.9 ± 0.6
Well-differentiated carcinoma (Grade I) (N = 12)	18.3 ± 2.4
Moderately differentiated carcinoma (Grade II) (N = 12)	36.2 ± 3.4
Poorly differentiated carcinoma (Grade III) (N = 12)	82.8 ± 6.6

*The cervical tissue extracts were first adjusted to identical concentrations (~1 mg/ml of tissue protein in 200 μl of solution A) and then immunoprecipitated by 20 μg of anti-kinase F_A/GSK-3α antibody, followed by kinase F_A/GSK-3α activity assay in the immunoprecipitates as described under Experimental Procedures. Data were expressed as means ± SD. Samples were categorized and graded according to the World Health Organization Histologic Classification of Invasive Squamous Cell Carcinoma.

human cervical carcinoma dedifferentiation and progression.

DISCUSSION

In this report, we demonstrate that protein kinase F_A/GSK-3α is consistently and statistically overexpressed and dysregulated in human cervical carcinoma as compared with normal controls. Based on computer-assisted sequence analysis of transcriptional factors and viral oncoprotein proteins [Suzuki, 1989], as well as analysis of site-specific protein phosphorylation both in vitro and in vivo [Moreno and Nurse, 1990; Pines and Hunter, 1990; Lin et al., 1991], it appears that proline-directed protein phosphorylation sites represent a unique structural motif that has been conserved and canalized as a major regulatory theme [Hall and Vulliet, 1991; Williams et al., 1992; Warburton et al., 1993]. In comparisons with cyclin-dependent cell division cycle control kinases [Vulliet et al., 1989; Hall and Vulliet, 1991], mitogen-activated protein kinases [Gonzalez et al., 1991; Mukhopadhyay et al., 1992], and stress-activated protein kinases [Kyriakis et al., 1994], protein kinase F_A/GSK-3α appeared to represent a particular member of proline-directed protein kinase family [Hemmings and Cohen, 1983; Vulliet et al., 1989; Ramakrishna et al., 1990; Yang et al., 1993; Yu and Yang, 1994a]. It has been proposed that overexpression, dysregulation, or viral subversion of some certain proline-directed protein kinases could be associated with neoplastic transformation and tumorigenesis. The results presented here that protein kinase F_A/GSK-3α, one of the members of proline-directed protein kinase family, is indeed greatly overexpressed and dysregulated in human cervical carcinoma, a virus-infected cancer, strongly support this notion. Since the protein levels and cellular activities of kinase F_A/GSK-3α appeared to be significantly and proportionally correlated with the degree of dedifferentiation and progression of human cervical carcinoma as presented here, together with the previous report that overexpression of kinase F_A/GSK-3α correlates with human thyroid cell dedifferentiation from normal, hyperplasia, adenoma to carcinoma stages [Lee et al., 1995], the results further support an association of kinase F_A/GSK-3α with human tumor cell dedifferentiation and/or progression. Since kinase F_A/GSK-3α activity is inversely proportional to the degree of tumor cell differentiation, this kinase may function as a negatively acting protein kinase influencing cellular differentiation and may, therefore, represent a newly described differentiation-blocking and/or dedifferentiation-promoting agent involved in promoting the progression of carcinoma cells. Kinase F_A/GSK-3α was assumed to be constitutively active due to the ready detection of the activity in resting cell extracts and has been concluded as a mitogen-inactivated protein kinase [Woodgett, 1991; Hughes et al., 1993; Woodgett et al., 1993]. However, as presented in this report that kinase F_A/GSK-3α could be activated up to ~1,600% of normal control level during human cervical carcinoma dedifferentiation/progression, the results point out that kinase F_A/GSK-3α may function as a mitogen-activated protein kinase and may not represent a constitutively active/mitogen-inactivated protein kinase as previously conceived.

From the clinical viewpoint, since the cellular levels and biological activities of protein kinase F_A/GSK-3α are significantly and proportionally correlated with the degree of human cervical carcinoma dedifferentiation/progression, this kinase may possibly be used as a specific marker protein for clinical diagnosis of the status of cervical cancer during pre- and postdiagnosis of the disease or as an indicator of early diagnosis

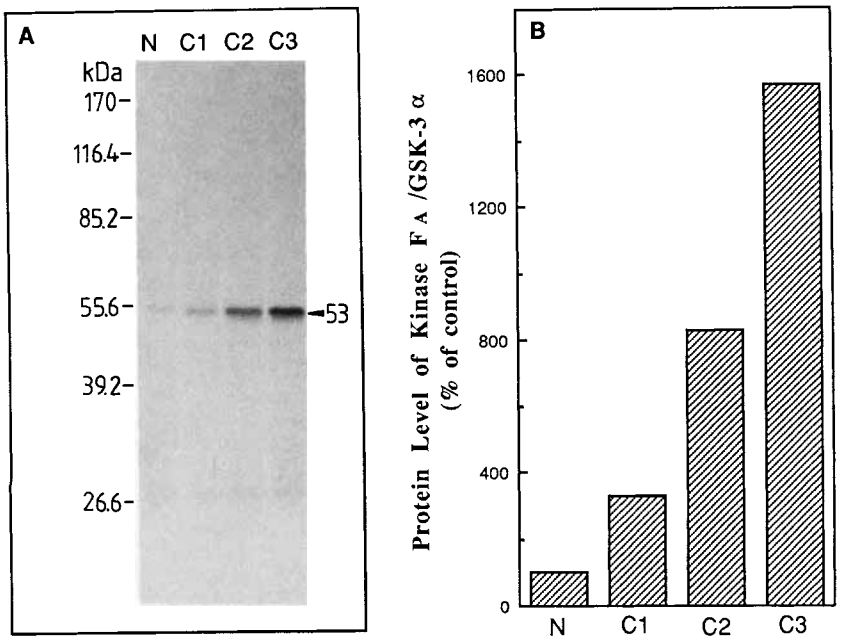

Fig. 2. Immunoblotting analysis of kinase F_A/GSK-3α from crude extracts of cervical tissues from normal control and different malignant lesions of the cervix. Crude extracts (~200 μg protein each) of the cervical tissues from normal control and different malignant lesions of the cervix were subjected to 10% SDS-PAGE followed by (**A**) immunoblotting with anti-kinase F_A/GSK-3α antibody and (**B**) quantification of the relative amount of kinase F_A/GSK-3α on the immunoblot as described under Experimental Procedures. *Lane N*, normal control; *lanes C1–C3*, cervical carcinoma at well-, moderately, and poorly differentiated stages, respectively. The immunoblot shown here demonstrates representative results from 4 experiments using 4 sets of 16 samples.

for screening patients that may have a potential tendency for human cervical cancer. This obviously presents an important issue deserving further investigation. Nevertheless, the present study clearly demonstrates that protein kinase F_A/GSK-3α (a possible regulator of the transcription factors/proto-oncogenes and a member of proline-directed protein kinase family) is consistently and statistically activated many-fold in human cervical carcinoma and the activation state of the kinase is proportionally correlated with the state of dedifferentiation and progression of the human cervical carcinoma.

ACKNOWLEDGMENTS

This work was supported by grants (CMRP-263 and CMRP-487) of Chang Gung Medical College of Taiwan, Republic of China. The work was also supported by grants NSC84-2332-B-007-001 and NSC84-2311-13-007-001 from National Science Council of Taiwan, Republic of China.

REFERENCES

Boyle WJ, Smeal T, Defize LHK, Angel P, Woodget JR, Karin M, Hunter T (1991): Activation of protein kinase C decrease phosphorylation of c-jun at sites that negatively regulate its DNA-binding activity. Cell 64:573–584.

de Groot RP, Auwerx J, Bourous M, Sassone-Corsi P (1992): Negative regulation of Jun/AP-1: Conserved function of glycogen synthase kinase 3 and the *Drosophila* kinase *shaggy*. Oncogene 7:841–847.

de Groot RP, den Hertog J, Vandenheede JR, Goris J, Sassone-Corsi P (1993): Multiple and cooperative phosphorylation events regulate the CREM activator function. EMBO J 12:3903–3911.

Dent P, Campbell DG, Hubbard MJ, Cohen P (1989): Multisite phosphorylation of the glycogen-binding subunit of protein phosphatase-1_G by cyclic AMP-dependent protein kinase and glycogen synthase kinase-3. FEBS Lett 248:67–72.

DePaoli-Roach AA (1984): Synergestic phosphorylation and activation of ATP.Mg-dependent phosphoprotein phosphatase by F_A/GSK-3 and casein kinase II. J Biol Chem 259:12144–12152.

Fiol CJ, Haesman JH, Wang Y, Roach PJ, Roeske RW, Kowulczuk M, DePaoli-Roach AA (1988): Phosphoserine as a recognition determinant for glycogen synthase kinase-3: Phosphorylation of a synthetic peptide based on the G-component of protein phosphatase-1. Arch Biochem Biophys 267:797–802.

Gonzalez F, Raden D, Davis R (1991): Identification of substrate recognition determinants for human ERK1 and ERK2 protein kinases. J Biol Chem 266:22159–22163.

Guan R, Khatra BS, Cohlberg JA (1991): Phosphorylation of bovine neurofilament proteins by protein kinase F_A (glycogen synthase kinase 3). J Biol Chem 266:8262–8267.

Hall F, Vulliet PR (1991): Proline-directed protein phosphorylation and cell cycle regulation. Curr Opin Cell Biol 3:176–184.

Hanger DP, Hughes K, Woodgett JR, Brion J-P, Anderton BH (1992): Glycogen synthase kinase-3 induces Alzheimer's disease-like phosphorylation of tau: Generation of paired helical filament epitopes and neuronal localisation of the kinase. Neurosci Lett 147:58–62.

Hemmings BA, Cohen P (1983): Glycogen synthase kinase-3 from rabbit skeletal muscle. Methods Enzymol 99:337–345.

Hemmings BA, Yellowlees D, Kernohan JC, Cohen P (1981): Purification of glycogen synthase kinase 3 from rabbit skeletal muscle. Copurification with the activating factor (F_A) of the (Mg.ATP) dependent protein phosphatase. Eur J Biochem 119:443–451.

Hemmings BA, Aitken A, Cohen P, Rymond M, Hofmann F (1982): Phosphorylation of the type-II regulatory subunit of cyclic AMP-dependent protein kinase by glycogen synthase kinase 3 and glycogen kinase 5. Eur J Biochem 127:473–481.

Hughes K, Ramakrishna S, Benjamin WB, Woodgett JR (1992): Identification of multifunctional ATP-citrate lyase kinase as the α-isoform of glycogen synthase kinase-3. Biochem J 288:309–314.

Hughes K, Nikolakaki E, Plyte SE, Totty NF, Woodgwtt JR (1993): Modulation of the glycogen synthase kinase-3 family by tyrosine phosphorylation. EMBO J 12:803–808.

Jurgensen S, Shacter-Noiman E, Huang CY, Chock PB, Yang S-D, Vandenheede JR, Merlevede W (1984): On the mechanism of activation of the ATP.Mg-dependent phosphoprotein phosphatase by kinase F_A. J Biol Chem 259: 5864–5879.

Kyriakis JM, Banerjee P, Nikolakaki E, Dai T, Rubie EA, Ahmad MF, Avruch J, Woodgett JR (1994): The stress-activated protein kinase subfamily of c-jun kinases. Nature 369:156–160.

Laemmli UK (1970): Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature 227:680–685.

Lee T-T, Ho Y-S, Yu J-S, Yang S-D (1995): Overexpression of cellular activity and protein level of protein kinase F_A/GSK-3α correlates with human thyroid tumor cell dedifferentiation. J Cell Biochem 59:108–116.

Lin B, Gruenwald S, Morla A, Lee W, Wang J (1991): Retinoblastoma cancer suppressor gene product is a substrate of the cell cycle regulator cdc2 kinase. EMBO J 10:13383–13390.

Mackie K, Sorkin BC, Nairn AC, Greengard P, Edelman GM, Cunningham BA (1989): Identification of 2 protein kinases that phosphorylate the neural cell-adhesion molecule, N-CAM. J Neurosci 9:1883–1896.

Mandelkow E-M, Drewes G, Biernat J, Gustke N, Van Lint J, Vandenheede JR, Mandelkow E (1992): Glycogen synthase kinase-3 and the Alzheimer-like state of microtubule-associated protein tau. FEBS Lett 314:315–321.

Moreno S, Nurse P (1990): Substrates for $p34^{cdc2}$: In vivo veritas? Cell 61:549–551.

Mukhopadhyay NK, Price DJ, Kyriakis JM, Pelech S, Sanghera J, Avruch J (1992): An array of insulin-activated, proline-directed serine/threonine protein kinases phosphorylate the S6 kinase. J Biol Chem 267:3325–3335.

Pines J, Hunter T (1990): $P34^{cdc2}$: The S and M kinase? New Biologist 2:389–401.

Plyte SE, Hughes K, Nikolakaki E, Pulverer BJ, Woodgett JR (1992): Glycogen synthase kinase-3: Functions in oncogenesis and development. Biochem Biophys Acta 1114: 147–162.

Ramakrishna S, D'Angelo G, Benjamin W (1990): Sequence of sites on ATP-citrate lyase and phosphatase inhibitor 2 phosphorylated by multifunctional protein kinase (a glycogen synthase kinase 3 like kinase). Biochemistry 29:7617–7624.

Suzuki M (1989): SPXX, a frequent sequence motif in gene regulatory proteins. J Mol Biol 207:61–84.

Taniuchi M, Johnson EM, Roach PJ, Lawrence JC Jr (1986): Phosphorylation of the nerve growth factor receptor proteins in sympathic neurons and PC12 cells. In vitro phosphorylation by the cAMP-independent protein kinase F_A/GSK-3. J Biol Chem 261:11786–11791.

Vandenheede JR, Yang S-D, Goris J, Merlevede W (1980): ATP.Mg-dependent protein phosphatase from rabbit muscle. Purification of the activating factor and its regulation as a bifunctional protein also displaying synthase kinase activity. J Biol Chem 255:11768–11774.

Vulliet PR, Hall FL, Mitchell JP, Hardie DG (1989): Identification of a novel proline-directed serine/threonine protein kinase in rat pheochromocytoma. J Biol Chem 264:16292–16298.

Wang QM, Park I-K, Fiol CJ, Roach PJ, Depaoli-Roach AA (1994): Isoform differences in substrate recognition by glycogen synthase kinase 3α and 3β in the phosphorylation of phosphatase inhibitor 2. Biochemistry 33:143–147.

Warburton D, Berndt N, Hall F (1993): "Signal Transduction in Lung Cells." Brody et al. (eds). New York: Marcel Dekker, Inc., pp 245–261.

Williams RT, Carbonaro-Hall DA, Hall FL (1992): Copurification of $p34^{cdc2}$/$p58^{cyclinA}$ proline-directed protein kinase and the retinoblastoma tumor susceptibility gene product: Interaction of an oncogenic serine/threonine protein kinase with a tumor-suppressor protein. Oncogene 7:423–432.

Woodgett JR (1990): Molecular cloning and expression of glycogen synthase kinase-3/Factor A. EMBO J 9:2431–2438.

Woodgett JR (1991): A common denominator linking glycogen metabolism, nuclear oncogenes and development. Trends Biochem Sci 16:177–181.

Woodgett JR, Plyte SE, Pulverer BJ, Mitchell JA, Hughes K (1993): Roles of glycogen synthase kinase-3 in signal transduction. Biochem Soc Trans 21:905–907.

Yang S-D (1986): Identification of the ATP.Mg-dependent protein phosphatase activator F_A as a myelin basic protein kinase in the brain. J Biol Chem 261:11786–11791.

Yang S-D (1991): Characteristics and regulation of the ATP.Mg-dependent protein phosphatase activating factor (protein kinase F_A). Adv Protein Phosphatases 6:133–157.

Yu J-S, Yang S-D (1993a): Identification and characterization of protein kinase F_A/glycogen synthase kinase 3 in clathrin-coated brain vesicles. J Neurochem 60:1714–1721.

Yu J-S, Yang S-D (1993b): Immunological and biochemical study on tissue and subcellular distributions of protein kinase F_A (an activating factor of ATP.Mg-dependent protein phosphatase): A simplified and efficient procedure for high quantity purification from brain. J Protein Chem 12:665–674.

Yu J-S, Yang S-D (1994a): Protein kinase F_A/glycogen synthase kinase-3 predominantly phosphorylates the in vivo site Thr^{97}-Pro in brain myelin basic protein. Evidence for Thr-Pro and Ser-Arg-X-X-Ser as consensus sequences motif. J Neurochem 62:1596–1603.

Yu J-S, Yang S-D (1994b): Okadaic acid, a serine/threonine phosphatase inhibitor, induces tyrosine dephosphorylation/inactivation of kinase F_A/GSK-3α in A431 cells. J Biol Chem 269:14341–14344.

Yu J-S, Yang S-D (1994c): Tyrosine dephosphorylation and concurrent inactivation of protein kinase F_A/GSK-3α by genistein in A431 cells. J Cell Biochem 56:131–141.

Yang S-D, Vandenheede JR, Goris J, Merlevede W (1980): ATP.Mg-dependent phosphorylase phosphatase from rabbit muscle. J Biol Chem 255:11759–11767.

Yang S-D, Liu J-S, Fong Y-L, Yu J-S, Tzen T-C (1987) Endogenous basic protein phosphatases in the brain myelin. J Neurochem 48:160–166.

Yang S-D, Yu J-S, Lai Y-G (1991): Identification and characterization of the ATP-Mg-dependent protein phosphatase activator (F_A) as a microtubule protein kinase in the brain. J Protein Chem 10:171–181.

Yang S-D, Song J-S, Yu J-S, Shiah S-G (1993): Protein kinase F_A/GSK-3 phosphorylates tau on Ser^{235}-Pro and Ser^{404}-Pro that are abnormally phosphorylated in Alzheimer's disease brain. J Neurochem 61:1742–1747.