Research Article

# The Role of Glycogen Synthase Kinase 3β in the Transformation of Epidermal Cells

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## Abstract

**Glycogen synthase kinase 3β (GSK3β) is a multifunctional serine/threonine kinase. We showed that the expression of GSK3β was drastically down-regulated in human cutaneous squamous cell carcinomas and basal cell carcinomas. Due to its negative regulation of many oncogenic proteins, we hypothesized that GSK3β may function as a tumor suppressor during the neoplastic transformation of epidermal cells. We tested this hypothesis using an *in vitro* model system, JB6 mouse epidermal cells. In response to epidermal growth factor (EGF) or 12-*O*-tetradecanoylphorbol-13-acetate (TPA), the promotion-sensitive JB6 P+ cells initiate neoplastic transformation, whereas the promotion-resistant JB6 P− cells do not.**

number of external signals (3). GSK3β activity is regul specific phosphorylation. Full activity of GSK3β gener phosphorylation on $Tyr^{216}$, and conversely, phosph $Ser^{9}$ inhibits GSK3β activity. GSK3β is a negative regul β-catenin signaling (1, 4). Because some oncogenic t factors [e.g., activator protein-1 (AP-1)] and proto-c (i.e., β-catenin) are putative GSK3β substrates for phos dependent inactivation (4), it has been hypothesized may interfere with cellular neoplastic transformation development. However, there are only limited studie the involvement of GSK3β in tumor development; are sometimes contradictory (5–8). The role of GS tumorigenesis remains unclear. Carcinogenesis is process that can be divided experimentally into t

**JB6 P− cells expressed much higher levels of GSK3β than JB6 P+ cells; JB7 cells, the transformed derivatives of JB6, had the least amount of GSK3β. The activity of GSK3β is negatively regulated by its phosphorylation at $Ser^9$. EGF and TPA induced strong $Ser^9$ phoshorylation in JB6 P+ cells, but phosphorylation was seen at a much lesser extent in JB6 P− cells. EGF and TPA-stimulated $Ser^9$ phosphorylation was mediated by phosphoinositide-3-kinase (PI3K)/Akt and protein kinase C (PKC) pathways. Inhibition of GSK3β activation significantly**

namely, initiation, promotion, and progression. I
associated with irreversible, carcinogen-mediated DN
In contrast, promotion is a reversible process in whi
increases in the rate of cell replication and/or alterat
expression. Progression represents the final gene
associated with the conversion of benign tumors
malignant cells. Skin cancer is the most common cance
(9). Our understanding of the mechanisms und
development and progression of skin tumors is still

## Materials and Methods

**Materials and cell cultures.** All antibodies except antiactin antibody were obtained from Cell Signaling Technology, Inc.. Antiactin antibody was purchased from Santa Cruz Biotechnology. PKC inhibitors (GF10203X or bisindolylmaleimide I and Go6976), PKA inhibitor (H89), GSK3β inhibitor (TDZD-8), and MEK1 inhibitor (PD98059) were purchased from Calbiochem. PI3K inhibitors (LY294002 and wortmannin), GSK3β inhibitor (SB216763), LiCl, and lactacystin were purchased from Sigma Chemical Co.. c-Jun-$NH_2$-kinase (JNK) inhibitor (D-JNKI) was purchased from Alexis Biochemicals.

JB6 P+ mouse epidermal cell line (Cl 41), JB6 P− and transformed JB7 cells were grown in EMEM containing 10% fetal bovine serum (FBS), 2 mmol/L L-glutamine, and 25 μg/mL gentamicin at 37°C with 5% $CO_2$. JB7 cells were derived from soft agar colonies of JB6 P+ treated with TPA for 3 weeks. These cells form colonies in soft agar and display tumorigenicity *in vivo*. The stable transfectants of JB6 P+ cells expressing AP-1-luciferase reporter (Cl 41 AP-1) have been previously described (17, 18).

**Human skin samples and immunohistochemical study of GSK3β.** Human skin tissues were obtained from surgical specimens at the Department of Dermatology, Xijing Hospital (Xi'an, China). The protocol for collecting human tissues was approved by the Ethical Committee of Xijing Hospital. The specimens were formalin fixed and paraffin embedded. The samples comprised of 31 primary cutaneous squamous cell carcinomas and 12 basal cell carcinomas. The median patient age was 61 years. Tumor diagnoses were established through pathologic evaluation of paraffin-embedded tissues stained with H&E. None of the patients received radiation or chemotherapy before the operation. Eight samples of histologically normal adult skin tissues were collected as controls.

at 37°C with an atmosphere of 5% $CO_2$ for 10 to 14 days, and of induced cell colonies was counted under a microsco containing eight or more cells were counted in four 0 randomly chosen with respect to distance from the center of the count was multiplied by the appropriate factor to giv number per well.

**Tumorigenicity in nude mice.** To evaluate *in vivo* tu 5-week-old male nude mice (BALB/c *nu/nu*, ~25 g; C Laboratories) were used. JB6 P+ cells and stable transfectan various GSK3β constructs were treated with EGF or TPA (0 or 6 days and then dissociated from monolayer cultures by t Cells were counted and centrifuged at 1,500 rpm for 5 min and in PBS. An aliquot of cells ($5 \times 10^6$ in 100 μL of PBS) that were EGF or TPA (0 or 10 ng/mL) was directly injected to both animals. One injection per flank was done for each mouse. E were used for each treatment group. Mice were maintained in free environment; food and water were given *ad libitum*. Seve the initial injections, the length ($L$) and width ($W$) of the s.c. were measured by calipers, and the tumor volume (TV) was described by Yaguchi et al. (20): $TV = 0.5 \times L \times W^2$. At th experiments, mice were sacrificed using a $CO_2$ chamber. An and all procedures followed the NIH Guide for the Care Laboratory Animals and were approved by the West Virgin Animal Care and Use Committee. Every effort was made t number of animals and their suffering.

**Measurement of AP-1 activity.** AP-1 transactivation epidermal cells was determined by assaying the activity of t reporter (17, 18). The assay accurately measures AP-1 tr (13, 18). Briefly, JB6 P+ cells expressing AP-1-luciferase reporte

Immunohistochemistry for GSK3β was done by the avidin-biotin indirect immunoperoxidase method. Briefly, 4-μm-thick sections were dewaxed, rehydrated, and incubated with 0.3% hydrogen peroxide for 30 min to block endogenous peroxidase activity. Sections were microwave treated in 0.01 mol/L citrate buffer (pH, 6.0) at 700 W for 10 min and rinsed with 0.01 mol/L TBS. Sections were incubated with normal horse serum for 20 min and then with primary antibodies (dilution 1:100) overnight at 4°C. After rinsing in TBS, sections were incubated with biotinylated secondary antibodies at room temperature for 30 min, followed by an avidin-biotin-peroxidase complex (Fisher Scientific) for 30 min. The reaction was

were cultured in 96-well plates and grown in a medium contair The plates were incubated at 37°C in a humidified atmos $CO_2$. Subconfluent cultures were maintained in a mediur 0.1% FBS for 24 h and treated with or without various pr inhibitors 30 min before exposure to EGF or TPA. After treatr protein was extracted with a 1× lysis buffer supplied in the lu kit (Promega), and luciferase activity was measured with luminometer (3010, Analytical Luminescence Laboratory). (luciferase activity) was calculated and expressed relative to t cultures.

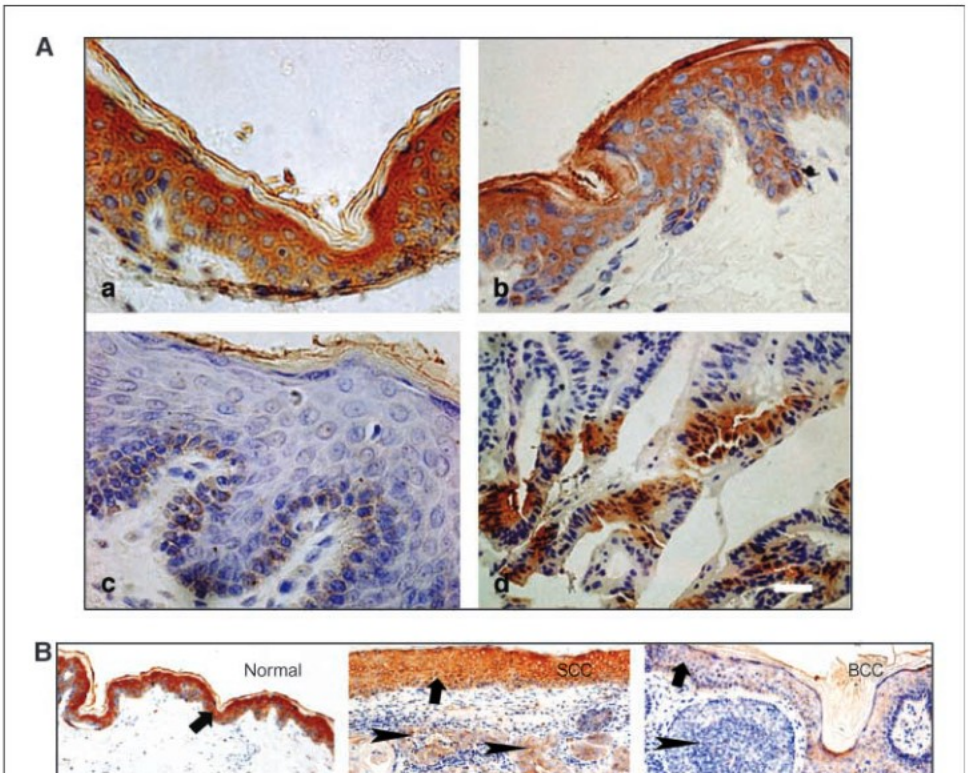
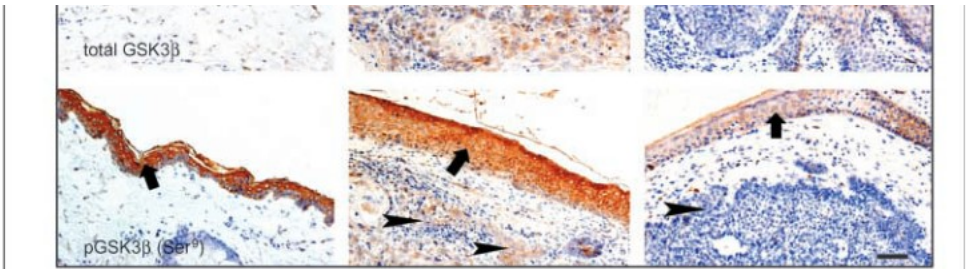
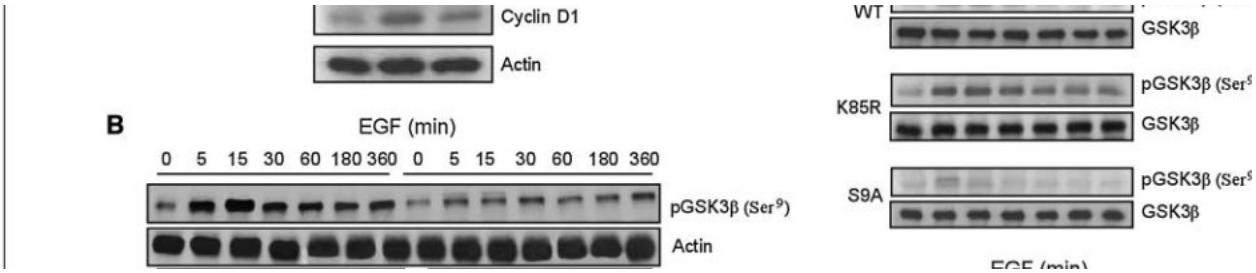
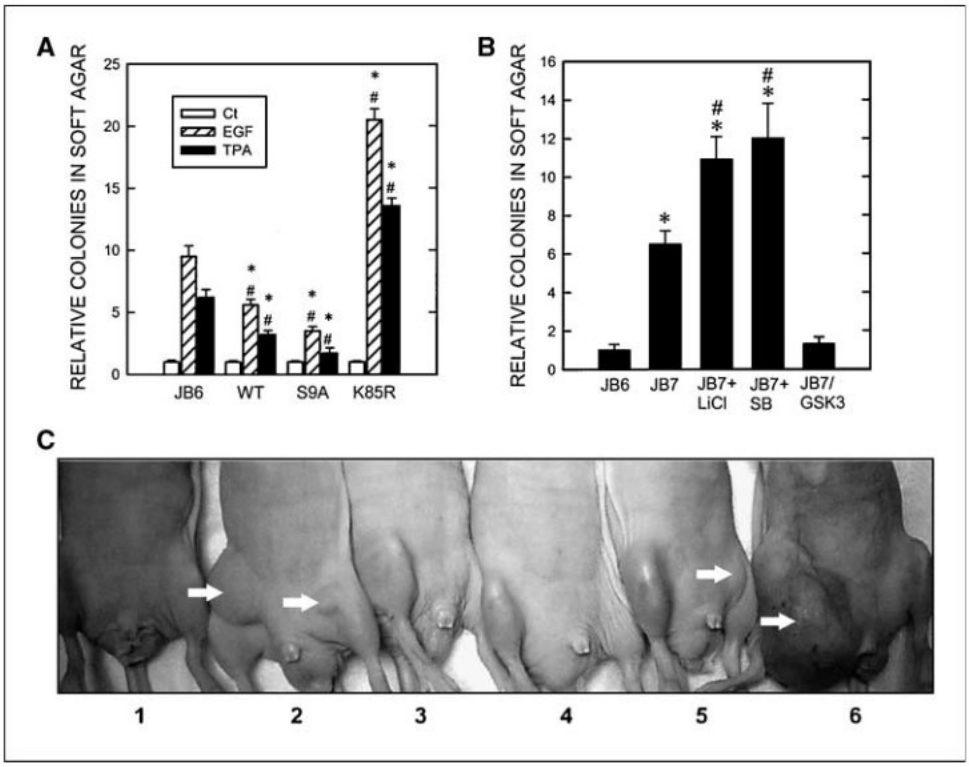



**Figure 1.** Immunohistocher
GSK3β expression in norma
tissues and tumor-bearing ti
expression of GSK3β and p
forms (Ser$^{9}$ and Tyr$^{216}$) was
paraffin-embedded normal h
tissues as described in Mate
Methods. *a*, expression of G
*b*, expression of phosphoryl
(Ser$^{9}$) [pGSK3β (Ser$^{9}$)]. *c*, e
pGSK3β (Tyr$^{216}$). *d*, human
tissues were used as a posi
immunostaining of pGSK3β
*B*, a representative microph
shows GSK3β and pGSK3β
immunostaining in tumor-be
tissues and histologically no
tissues obtained from age- a
sex-matched subjects. *SCC*
cell carcinomas; *BCC*, basa
carcinomas. *Arrows*, normal
*arrowheads*, tumor tissues.


total GSK3β
pGSK3β (Ser9)

The effect of GSK3β on the transformation of JB6 P+ cells was first determined by anchorage-independent growth in soft agar. As shown in Fig. 3*A*, EGF and TPA stimulated the formation of cell colonies in soft agar. EGF- and TPA-induced cell colonies were significantly suppressed by the overexpression of WT and S9A GSK3β. In contrast, EGF- and TPA-induced anchorage-independent growth was drastically enhanced by the over-expression of K85R mutant. It was noted that JB6 P+ cells expressing K85R formed some small cell colonies (contained less than eight cells) in the absence of EGF and TPA. These small cell colonies were not scored. The results suggested that GSK3β was a negative regulator of cell transformation. JB7 cells formed colonies in soft agar (Fig. 3*B*). Two inhibitors of GSK3β (LiCl and SB216763) significantly enhanced anchorage-independent growth of JB7 cells. In contrast, overexpression of GSK3β in JB7 cells suppressed anchorage-independent growth (Fig. 3*B*). To further assess the role of GSK3β in tumorigenicity, we injected EGF- or TPA-exposed JB6 P+ cells expressing WT, S9A, or K85R GSK3β to nude mice and evaluated the formation of s.c. tumors. Cells were injected to both flanks for each mouse, and each flank received one injection. There were eight animals injection group. The number and the size of s.c. tu measured 7 weeks following initial injection. EGF exposed JB6 P+ cells expressing control vectors tumors in nude mice (Fig. 3*C*). For example, eig received injections of EGF-exposed JB6 P+ cells (ea received two injections; one in each flank), a total of formed, and the average volume of each tumor was $mm^3$ (Table 1). However, in the eight animals injected exposed cells expressing WT or S9A GSK3β, only thr tumors formed, respectively. In addition, the average tumors was significantly smaller. The tumor volumes injected with cells expressing WT or S9A GSK3β wer and 478 ± 81 $mm^3$, respectively. In contrast, EGF-ex overexpressing K85R showed enhanced tumorigenici mice; a total of 14 tumors formed, and the average each tumor was 3756 ± 279 $mm^3$ (Table 1). Similar r obtained with the treatment of TPA (Table 1). It was three small tumors (368 ± 65 $mm^3$) formed in the nud received an injection of cells expressing K85R tha


Cyclin D1
Actin
B
EGF (min)
0 5 15 30 60 180 360 0 5 15 30 60 180 360
pGSK3β (Ser9)
Actin
WT
GSK3β
K85R
pGSK3β (Ser9)
GSK3β
S9A
pGSK3β (Ser9)
GSK3β


A
RELATIVE COLONIES IN SOFT AGAR
Ct
EGF
TPA
JB6
WT
S9A
K85R
B
RELATIVE COLONIES IN SOFT AGAR
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JB7+ LiCl
JB7+ SB
JB7/ GSK3
C
1
2
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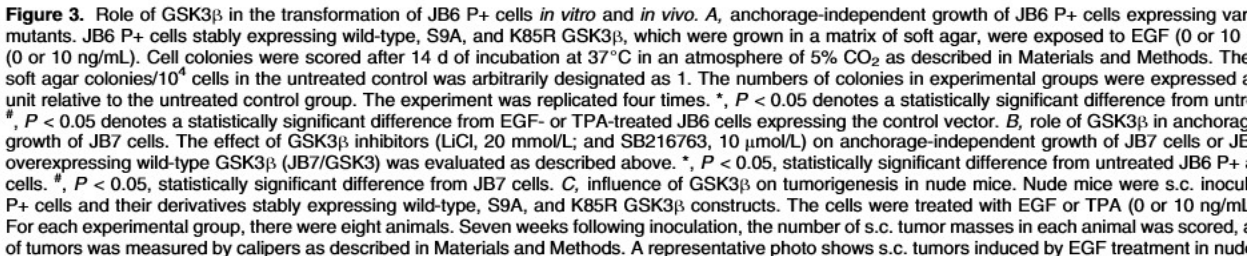
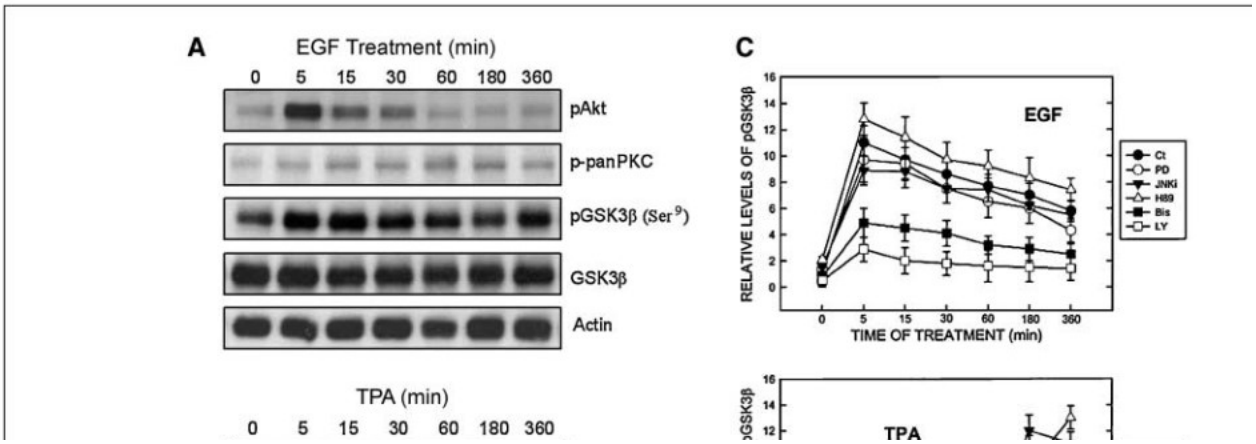
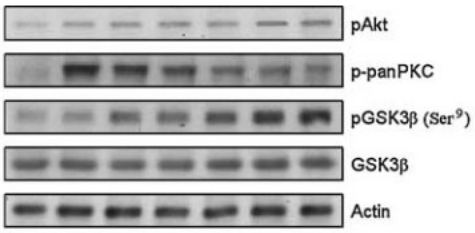


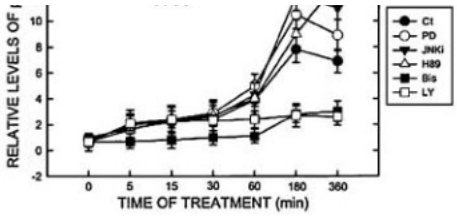
**Figure 3.** Role of GSK3β in the transformation of JB6 P+ cells *in vitro* and *in vivo*. *A,* anchorage-independent growth of JB6 P+ cells expressing var mutants. JB6 P+ cells stably expressing wild-type, S9A, and K85R GSK3β, which were grown in a matrix of soft agar, were exposed to EGF (0 or 10 (0 or 10 ng/mL). Cell colonies were scored after 14 d of incubation at 37°C in an atmosphere of 5% $CO_2$ as described in Materials and Methods. The soft agar colonies/$10^4$ cells in the untreated control was arbitrarily designated as 1. The numbers of colonies in experimental groups were expressed a unit relative to the untreated control group. The experiment was replicated four times. *, $P < 0.05$ denotes a statistically significant difference from untr #, $P < 0.05$ denotes a statistically significant difference from EGF- or TPA-treated JB6 cells expressing the control vector. *B,* role of GSK3β in anchorag growth of JB7 cells. The effect of GSK3β inhibitors (LiCl, 20 mmol/L; and SB216763, 10 μmol/L) on anchorage-independent growth of JB7 cells or JE overexpressing wild-type GSK3β (JB7/GSK3) was evaluated as described above. *, $P < 0.05$, statistically significant difference from untreated JB6 P+ cells. #, $P < 0.05$, statistically significant difference from JB7 cells. *C,* influence of GSK3β on tumorigenesis in nude mice. Nude mice were s.c. inocul P+ cells and their derivatives stably expressing wild-type, S9A, and K85R GSK3β constructs. The cells were treated with EGF or TPA (0 or 10 ng/mL For each experimental group, there were eight animals. Seven weeks following inoculation, the number of s.c. tumor masses in each animal was scored, a of tumors was measured by calipers as described in Materials and Methods. A representative photo shows s.c. tumors induced by EGF treatment in nud

different. EGF induced a rapid and strong phosphorylation of GSK3β (Ser$^9$), the maximal phosphorylation occurred between 5 and 15 min after EGF treatment. TPA-mediated phosphorylation of GSK3β (Ser$^9$) was gradual; it became evident at 15 min, and maximal phosphorylation occurred at 3 to 6 h. Inhibitors of PI3K blocked EGF-stimulated pGSK3β (Ser$^9$) in JB6 P+ cells; an inhibitor of PKC also decreased EGF-stimulated pGSK3β (Ser$^9$), but to a lesser extent (Fig. 4*B* and *C*), suggesting that the PI3K/Akt pathway played a major role in EGF regulation of pGSK3β (Ser$^9$). On the other hand, inhibitor of PKC was more effective than the PI3K inhibitor in down-regulating TPA-induced pGSK3β inhibitors for MEK1, JNK, and PKA had little effect o TPA-induced pGSK3β (Ser$^9$; Fig. 4*B* and *C*). These resul that PI3K/Akt and PKC mediated EGF- and TPA-induc (Ser$^9$), although the profiles of regulation were differe

Like PKC and PI3K/Akt, ERKs are critical regula transformation of JB6 cells (13). MEK1 inhibitor (PD98 affect EGF- and TPA-mediated pGSK3β (Ser$^9$; Fig. suggesting that ERK was not involved in GSK3β ina JB6 cells. Reversely, GSK3β may inhibit ERK activati




pAkt
p-panPKC
pGSK3β (Ser9)
GSK3β
Actin


RELATIVE LEVELS OF
TIME OF TREATMENT (min)
0
5
15
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D EGF (min)

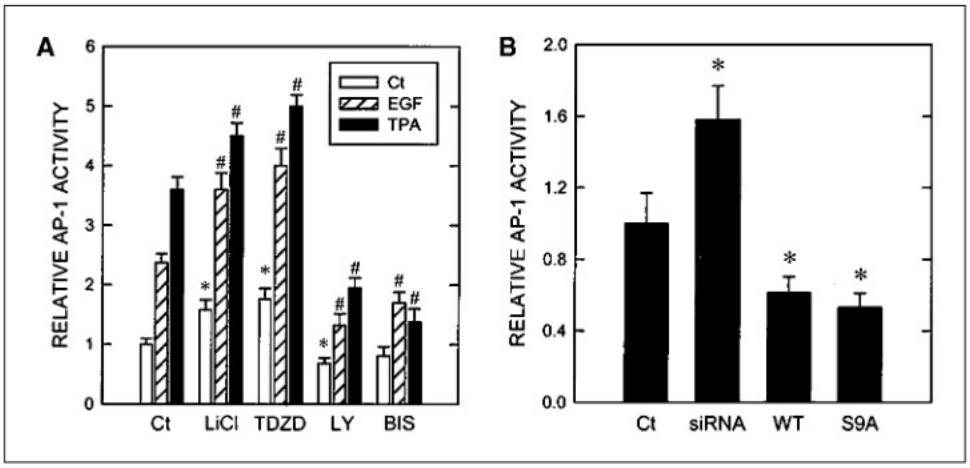


**Figure 5.** Role of GSK3β in AP-1 transactivation. *A,* JB6 P+ epidermal cells stably expressing AP-1 luciferase reporter were pretreated with two GSI LiCl (20 mmol/L) and TDZD8 (10 μmol/L) or PI3K inhibitor (*LY*, 10 μmol/L) and PKC inhibitor (*Bis*, 1 μmol/L) for 30 min and then exposed to EGF (10 TPA (10 ng/mL) for 12 h. The activity of AP-1 was measured by a luciferase assay as described in Materials and Methods. The activity of AP-1 was expres untreated cultures. The experiment was replicated thrice. *, $P < 0.05$, statistically significant difference from untreated JB6 P+ cells. #, $P < 0.05$, statistic difference from paired EGF- or TPA-treated JB6 cells. *B,* JB6 P+ epidermal cells stably expressing AP-1 luciferase reporter were transfected with eith S9A-mutated GSK3β constructs or an siRNA for GSK3β for 48 h. The activity of AP-1 was measured as described above. The experiment was replic *, $P < 0.05$, statistically significant difference from cells transfected with an empty vector.

therefore sought to determine whether GSK3β affected ERK activation in JB6 P+ cells. As shown in Fig. 4*D*, EGF elicited similar

subjects. The decreased immunostaining for pGSK3β results from the down-regulation of total GSK3β expres

phosphorylation of ERK in cells expressing W1, S9A, and K85R GSK3β; furthermore, two inhibitors of GSK3β (LiCl and TDZD8) failed to affect ERK phosphorylation (data not shown), indicating that GSK3β was not involved in ERK activation.

**GSK3β is involved in the regulation of AP-1.** It has been shown that AP-1 activity is essential for the transformation of JB6 P+ cells (12, 13, 23). We sought to determine whether GSK3β regulates the activation of AP-1. As shown in Fig. 5*A*, two inhibitors of GSK3β (LiCl and TDZD8) stimulated the basal as well as EGF- and TPA-induced activation of AP-1. In contrast, inhibitors for PI3K

epidermal (JB6) cells have been extensively used as model for studying the promotion of neoplastic tra (10, 12–15). Consistent with the observations in l tissues, the expression levels of GSK3β in JB6 cells are the stage or potential of cell transformation. JB transformed derivatives of JB6, have the least amoun whereas promotion-resistant JB6 P− cells express the h of GSK3β; the levels of GSK3β in promotion-sensitive are intermediate. In addition, tumor promoters EG induce strong phosphorylation of GSK3β at $Ser^9$ in

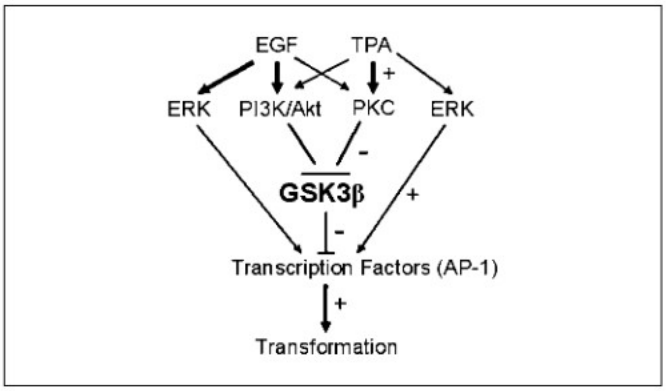


**Figure 6.** Diagram of the role of GSK3β in skin tumorigenesis. EGF and TPA activate PI3K/Akt and PKC, which induce phosphorylation of GSK3β ($Ser^9$) and inactivate GSK3β. Inactivation of GSK3β stimulates oncogenic transcription factors, such as AP-1. EGF and TPA can also activate AP-1 through the MEK1/Erk pathway. Hyperactivity of AP-1 promotes skin tumorigenesis.

apoptosis, and differentiation (28). The mouse epidermal multistage carcinogenesis model provides a well-defined system for examining the transformation of squamous epithelial cells to benign squamous papillomas and their subsequent progression into squamous cell carcinomas (29). With this carcinogenesis model system, a number of studies show that AP-1 activity is required for skin tumorigenesis as well as malignant transforma-

addition to negatively regulating AP-1 activity, GSK3β known inhibitor for Wnt/β-catenin signaling (1). H of the Wnt/β-catenin signaling pathway is associate development of a number of tumors as well as m (35–39). In terms of skin tumors, Wnt signaling implicated in the development and progression of (40). From the present study, however, it is not clear wh β-catenin signaling is also involved in GSK3β-induced of skin tumorigenesis.

Other studies also support a role of GSK3β as suppressor." For example, expression of a kinase-inactiv adult mouse mammary glands promotes mammary tur indicating that antagonism of GSK3β activity is on mammary epithelial cells (6). In a mouse epidermal carcinogenesis model, Leis et al. (41) show a dramatic pGSK3β ($Ser^9$) in late papillomas and squamous cell Furthermore, a significant decrease in pGSK3β ($Tyr^{216}$) in squamous cell carcinoma samples (41), indicating tion of GSK3β during mouse skin carcinogenesis. Tog observations support the notion that GSK3β is a negati of skin tumorigenesis; down-regulation or inactivation oncogenic for epidermal cells.

The mechanisms underlying skin tumorigenesis are c involve interactions among multiple signal cascades transcription factors. Our study clearly shows that G important component in the cascades, and modulatio

tion (30–33). These findings are confirmed by *in vitro* studies that show that AP-1 activity is essential for the transformation of JB6 P+ cells (12, 13, 23). Three signaling pathways, namely, PI3K/Akt, PKC, and MEK1/Erk, have been shown to regulate EGF- and TPA-induced AP-1 transactivation and transformation of JB6 P+ cells (13, 14, 23). We show here that PI3K/Akt and PKC are upstream of GSK3β, and both EGF- and TPA-stimulated pGSK3β ($Ser^9$) is mediated by PI3K/Akt and PKC. Thus, suppressing GSK3β activity is one of the mechanisms for EGF- and TPA-induced AP-1

expression/activity is sufficient to alter the tran
potential of epidermal cells. Thus, GSK3β is a target for
prevention/intervention strategies. GSK3β has emer
attractive therapeutic target for the treatment o
neurologic diseases such as Alzheimer's and stroke. I
been used as a mood stabilizer to treat bipolar mood d
other inhibitors of GSK3β have entered clinical trials f
The potential role of these inhibitors in tumorigenesis
considered.

15. Nomura M, Ichimatsu D, Moritani S, et al. Inhibition of epidermal growth factor-induced cell transformation and Akt activation by caffeine. Mol Carcinog 2005;44: 67–76.
16. Dhar A, Young MR, Colburn NH. The role of AP-1, NF-κB and ROS/NOS in skin carcinogenesis: the JB6 model is predictive. Mol Cell Biochem 2002;234–235: 185–93.
17. Ma C, Wang J, Luo J. Exposure to asphalt fumes activates activator protein-1 through the phosphatidylinositol 3-kinase/Akt signaling pathway in mouse epidermal cells. J Biol Chem 2003;278: 44265–72.
18. Ma C, Wang J, Luo J. Activation of nuclear factor κB by diesel exhaust particles in mouse epidermal cells through phosphatidylinositol 3-kinase/Akt signaling pathway. Biochem Pharmacol 2004;67:1975–83.
19. Chen G, Ma C, Bower KA, Ke Z, Luo J. Interaction between RAX and PKR modulates the effect of ethanol on protein synthesis and survival of neurons. J Biol Chem 2006;281:15909–15.
20. Yaguchi S, Fukui Y, Koshimizu I, et al. Antitumor activity of ZSTK474, a new phosphatidylinositol 3-kinase inhibitor. J Natl Cancer Inst 2006;98:545–56.
21. Germain D, Russell A, Thompson A, Hendley J. Ubiquitination of free cyclin D1 is independent of phosphorylation on threonine 286. J Biol Chem 2000;275: 12074–9.
22. Le Floch N, Rivat C, De Wever O, et al. The proinvasive activity of Wnt-2 is mediated through a noncanonical Wnt pathway coupled to GSK-3β and c-Jun/AP-1 signaling. FASEB J 2005;19:144–6.
23. Dong Z, Huang C, Ma WY. PI-3 kinase in signal transduction, cell transformation, and as a target for chemoprevention of cancer. Anticancer Res 1999;19: 3743–7.
24. Wang Q, Zhou Y, Wang X, Evers BM. Glycogen synthase kinase-3 is a negative regulator of extracellular signal-regulated kinase. Oncogene 2006;25: 43–50.
25. Mazor M, Kawano Y, Zhu H, Waxman J, Kypta RM. Inhibition of glycogen synthase kinase-3 represses androgen receptor activity and prostate cancer cell growth. Oncogene 2004;23:7882–92.
26. Nikolakaki E, Coffer PJ, Hemelsoet R, Woodgett JR, Defize LH. Glycogen synthase kinase 3 phosphorylates Jun family members *in vitro* and negatively regulates their transactivating potential in intact cells. Oncogene 1993;8:833–40.
27. Troussard AA, Tan C, Yoganathan TN, Dedhar S. Cell-extracellular matrix interactions stimulate the AP-1 transcription factor in an integrin-linked kinase- and glycogen synthase kinase 3-dependent manner. Mol Cell Biol 1999;19:7420–7.
28. Eferl R, Wagner EF. AP-1: a double-edged sword in tumorigenesis. Nat Rev Cancer 2003;3:859–68.
29. Dlugosz A, Merlino G, Yuspa SH. Progress in cutaneous cancer research. J Investig Dermatol Symp Proc 2002;7:17–26.
30. Saez E, Rutberg SE, Mueller E, et al. c-Fos is required for malignant progression of skin tumors. Cell 1995;82: 721–32.
31. Thompson EJ, MacGowan J, Young MR, Colburn N, Bowden GT. A dominant negative c-jun specifically blocks okadaic acid-induced skin tur Cancer Res 2002;62:3044–7.
32. Young MR, Li JJ, Rincon M, et al. T demonstrate AP-1 (activator protein-1) is required for tumor promotion. Proc U S A 1999;96:9827–32.
33. Zenz R, Scheuch H, Martin P, et al. eyelid closure and skin tumor develo EGFR signaling. Dev Cell 2003;4:879–89.
34. Ding Q, Xia W, Liu JC, et al. Erk assc primes GSK-3β for its inactivation resu lation of β-catenin. Mol Cell 2005;19:15
35. Behrens J, Lustig B. The Wnt tumorigenesis. Int J Dev Biol 2004;48:47
36. Dihlmann S, von Knebel Doeberi catenin–pathway as a molecular target cancer therapeutics. Int J Cancer 2005;1
37. Ilyas M. Wnt signalling and the mecl tumour development. J Pathol 2005;205:
38. Karim R, Tse G, Putti T, Scolyer significance of the Wnt pathway in th human cancers. Pathology 2004;36:120–
39. Luu HH, Zhang R, Haydon RC, et al. signaling pathway as a novel cancer dr Cancer Drug Targets 2004;4:653–71.
40. Weeraratna AT. A Wnt-er wonderl plexity of Wnt signaling in melanoma. C sis Rev 2005;24:237–50.
41. Leis H, Segrelles C, Ruiz S, Santos Expression, localization, and activity o thase kinase 3β during mouse skin tun Carcinog 2002;35:180–5.

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