Mol. Cells *27*, 15-19
DOI/10.1007/s10059-009-0001-7

Molecules and Cells

Regulation of Notch1/NICD and Hes1 Expressions by GSK-3α/β

Yun Hye Jin, Hangun Kim, Minsoo Oh, Hyunkyung Ki, and Kwonseop Kim*

Notch signaling is controlled at multiple levels. In particular, stabilized Notch receptor activation directly affects the transcriptional activations of Notch target genes. Although some progress has been made in terms of defining the regulatory mechanism that alters Notch stability, it has not been determined whether Notch1/NICD stability is regulated by GSK-3α. Here, we show that Notch1/NICD levels are significantly regulated by GSK-3β and by GSK-3α. Treatment with LiCl (a specific GSK-3 inhibitor) or the overexpression of the kinase-inactive forms of GSK-3α/β significantly increased Notch1/NICD levels. Endogenous NICD levels were also increased by either GSK-3α/β- or GSK-3α-specific siRNA. Furthermore, it was found that GSK-3α binds to Notch1. Deletion analysis showed that at least three Thr residues in Notch1 (Thr-1851, 2123, and 2125) are critical for its response to LiCl, which increased not only the transcriptional activity of endogenous NICD but also Hes1 mRNA levels. Taken together, our results indicate that GSK-3α is a negative regulator of Notch1/NICD.

INTRODUCTION

Notch is a single pass transmembrane receptor that fundamentally determines cell fate. Notch receptor, which is formed as a heterodimer in the plasma membrane by furin-like protease, is activated by interacting with membrane-bound ligands in neighboring cells (Logeat et al., 1998). Moreover, sequential cleavages by TACE and γ-secretase complex can release Notch1 intracellular domain (NICD) from the membrane (Brou et al., 2000), which then translocates to the nucleus where it interacts with CSL (referred to as RBP-Jkappa or CBF-1 in mammals, and suppressor of Hairless (Su(H)) in *Drosophila*) (Kopan, 2002). Interaction between NICD and CSL induces the expressions of HES and Hes-related repressor (HERP) proteins, two families of basic helix-loop-helix transcription factors (Iso et al., 2003). Furthermore, Notch signaling is regulated at multiple levels, which include ligand internalization and receptor modification, and several studies have shown that various proteins are involved in the regulation of the activities and levels of Notch and NICD.

Glycogen synthase kinase-3 (GSK-3) is a serine/threonine kinase, and a regulatory component in several cellular signal transduction pathways. Moreover, GSK-3 has two isoforms, GSK-3α and GSK-3β (Woodgett, 1990), which share high amino acid homology in their kinase domains. In resting cells, GSK-3 is normally active, i.e., hyperphosphorylated at Tyr-279 of GSK-3α and Tyr-216 of GSK-3β (both are located in the kinase domain) (Hughes et al., 1993; Wang et al., 1994). Numerous cellular stimuli cause the inactivation of GSK-3 via the phosphorylation of GSK-3α at Ser-21 and of GSK-3β at Ser-9, which are located in the Gly-rich domain (Cross et al., 1995). Although GSK-3α and GSK-3β are activated in the same manner, they are functionally distinct (Lee et al., 2007). Because GSK-3 can phosphorylate a wide spectrum of substrates, including various transcription factors and signal transcriptional molecules, its dysregulation can induce various cancers, mood disorders, and Alzheimer disease (Goto et al., 2002; Grimes and Jope, 2001; Liao et al., 2004).

Findings concerning the effects of GSK-3 on the stabilities of Notch1 and NICD are conflicting. For example, it has been reported that Notch2 phosphorylation by GSK-3β downregulates its activity (Espinosa et al., 2003), and on the other hand, another study found that GSK-3β positively regulates mammalian Notch1 signaling (Foltz et al., 2002). The majority of studies conducted on the relation between Notch and GSK-3 have focused on GSK-3β, and although it is known that the two GSK-3 isoforms have different functions, the role of GSK-3α is poorly understood. In particular, it has not been determined whether Notch1/NICD is regulated by GSK-3α. In the present study, we used LiCl to specifically inhibit GSK-3, and transfected wild type MEFs with wild-type GSK-3 isoforms or their dominant negative mutants (kinase-inactive forms) to determine whether Notch1/ NICD stability and NICD-dependent transactivation are affected by GSK-3α/β. Here, we present immunoprecipitation-based evidence that Notch1 and GSK-3α interact, and demonstrate that Notch1 levels and transcriptional activity are negatively regulated by GSK-3α/β via direct binding. We also show that the expression of Hes1 (a gene known to be targeted by NICD) is regulated by GSK-3α/β at the transcriptional level. Furthermore, we suggest for the first time that GSK-3α (like GSK-3β) is a negative regulator of the Notch signaling pathway.

College of Pharmacy and Research Institute of Drug Development, Chonnam National University, Gwangju 500-757, Korea
*Correspondence: koskim@chonnam.ac.kr

Received April 15, 2008; revised October 7, 2008; accepted October 13, 2008; published online October 13, 2008

Keyword: GSK-3, Hes1, LiCl, NICD, Notch1

MATERIALS AND METHODS

Plasmid construction

The C-terminal myc tagged constitutively active form of Notch1 (ΔEN1-myc) and Flag tagged NICD were generously donated by Raphael Kopan (Washington University, USA), and HA-tagged GSK-3β wild type and kinase-inactive constructs by Kang-Yeol Choi (Yonsei University, KOREA). Myc tagged Notch1 deletion constructs and HA tagged GSK-3α wild type and kinase-inactive constructs were generated in a CMV promoter-derived mammalian expression vector (pCS4-3Myc, -3HA) by polymerase chain reaction (PCR) amplification.

Antibodies and reagents

Antibodies against GFP (Clontech, BD Biosciences) and NICD (Cell Signaling Technology, Inc.) and GSK-3α/β (Abcam) were used, and Myc and HA epitope were detected using media from 9E10 and 12CA5 hybridomas. LiCl was purchased from Sigma, and siRNA for control (#6201), GSK-3α (#6312), and GSK-3α/β (#6301) were from Cell Signaling Technology, Inc.

Cell culture, transfection and reporter assays

All tissue culture media and antibiotics were purchased from Invitrogen. Mouse embryonic fibroblast (MEF) cells and 293 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C under a 5% CO_2 atmosphere. Transient transfections were performed using the calcium phosphate method by using Lipofectamine Plus reagent (Invitrogen), according to the manufacturer's instructions. siRNA was transfected by Lipofectamine Plus reagent. For reporter assays, cells were plated into 24-well plates 1 day before transfection. At 24 or 36 h posttransfection, cell lysates were analyzed for luciferase activity using luciferase reporter assay kits (Promega). pCMV β-galactosidase plasmid was used as an internal control to normalize transfection efficiencies. In terms of LiCl treatment, LiCl was added at the indicated concentrations in Figs. 1 and 4B to medium 3 h after transfection. To determine protein stability, cells were treated with cycloheximide (CHX, 40 μg/ml, Sigma) and then harvested at indicated time point.

Immunoprecipitation and immunoblot analysis

After transfection, cells were lysed in ice-cold lysis buffer (25 mM HEPES, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.25% Na deoxycholate, 10% glycerol, 25 mM NaF, 1 mM EDTA, 1 mM Na_3VO_4, 250 μM phenylmethylsulfonylfluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin). For immunoprecipitation analysis, lysates were centrifuged and resulting supernatants were immunoprecipitated with appropriate antibodies and pulled down with protein A- or protein G-Sepharose beads (Amersham Biosciences). Beads were then washed thoroughly, and bound proteins were resolved by SDS-PAGE and immunoblotted [immunoblot analysis was performed as described previously (Kim et al., 2005)]. Protein bands were quantified using TINA 2.09 software (Raytest).

RNA extraction and RT-PCR

The total RNA was isolated from cells using Tri reagent (as recommended by the manufacturer), and RNA yields and purities were estimated spectrophotometrically using $A_{260/280}$ ratios. cDNA was prepared using AccuPower® RT PreMix and oligo $(dT)_{20}$ primers from 1 μg of total RNA. The primers used in PCR analysis were as follows; Hes1 forward, 5′-GCA CAG AAA GTC ATC AAA GCC-3′; reverse, 5′-TTG ATC TGG GTC ATG CAG TTG-3′; β-actin forward, 5′-TCA CCC ACA CTG TGC CCA TCT ACG A; reverse, TGA TGA CCT GGC CGT CAG GCA GCT C-3′. β-actin was used as an internal control.

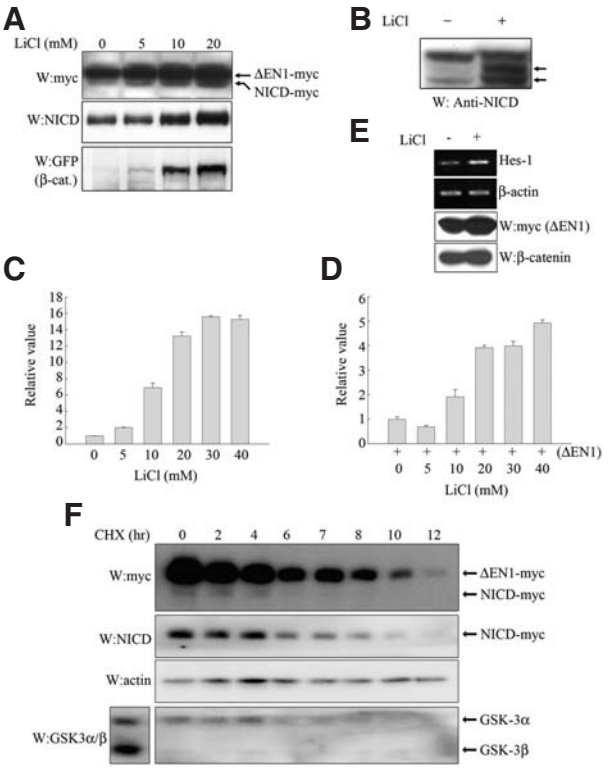

Fig. 1. Inhibition of GSK-3 activity increases the level and transcriptional activity of Notch1/NICD. (A) LiCl (a specific inhibitor of GSK-3) increased the levels of Notch1 and NICD. MEF cells were transfected with ΔEN1-myc or GFP-β-catenin and then treated with different concentrations of LiCl, as indicated. The expressions of Notch1 or NICD were followed using anti-myc (top panel) or anti-NICD Abs (middle panel), respectively, and β-catenin expression was analyzed using anti-GFP Ab (bottom panel). (B) LiCl increased the level of endogenous NICD. 293 HEK cells were treated with various concentrations of LiCl, and levels of endogenous NICD were examined using anti-NICD Ab. The arrow indicates endogenous NICD. (C) LiCl treatment increased NICD-dependent CSL promoter activity; CSL promoter reporter contains 4 copies of a CSL binding consensus sequence. The 293 HEK cells were transfected with CSL reporter construct and then treated with LiCl. Relative luciferase activities and their SDs are shown. All experiments were performed at least twice. (D) LiCl increased exogenous NICD-dependent CSL promoter activity (other than transfecting the cell lines with ΔEN1 construct exogenously, the procedure and analysis used were identical to those of panel C). (E) LiCl increased the mRNA levels of Hes1 and of Notch1 target genes. MEF cells were transfected with ΔEN1-myc, and total RNA was extracted from lysates. Reverse transcriptase PCR (RT-PCR) was performed using Hes1 primer and cDNA from 1 μg of total RNA. β-actin was used as a RT-PCR control. (F) The stability of Notch1 and NICD was correlated in GSK-3β -/- cells. GSK-3β -/- MEF cells were transfected with ΔEN1-myc and then were treated with cycloheximide (40 μg/ml) and harvested at the indicated time. Actin was used as a loading control.

RESULTS

Notch1 receptor and NICD levels are regulated by GSK-3α/β

GSK-3 is involved in the protein degradation pathway, in which

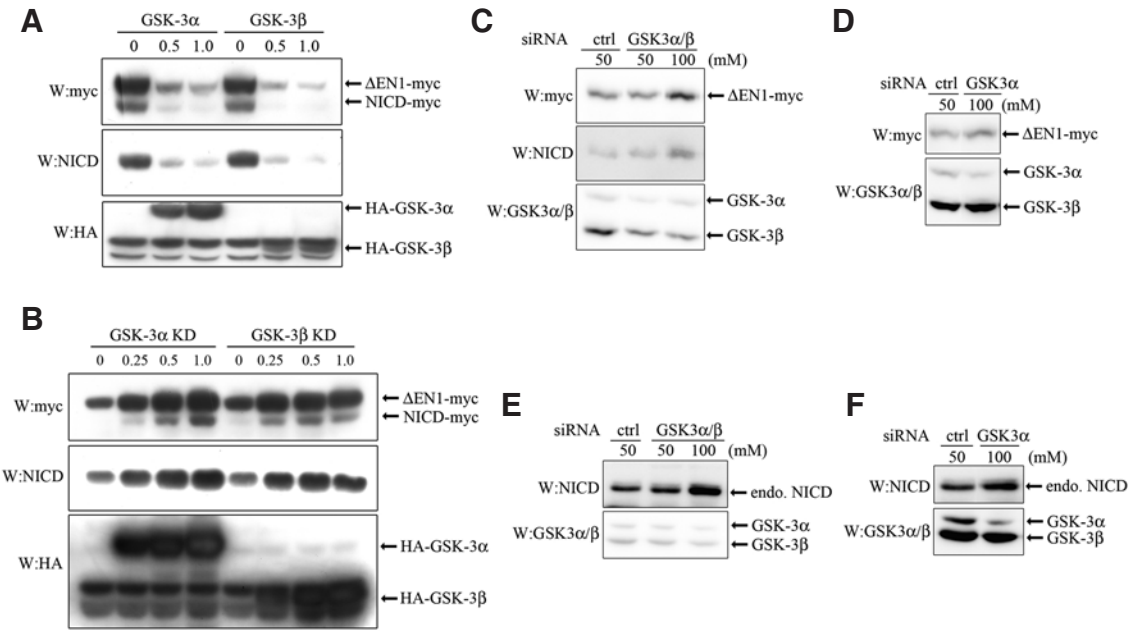

Fig. 2. GSK-3 affects the level of Notch1/NICD. (A) Notch1 levels were reduced by GSK-3α or -3β. MEF cells were transfected with ΔEN1-myc with HA-tagged GSK-3α or GSK-3β plasmids, as indicated. Notch1 and NICD levels were determined using anti-myc (top panel) or anti-NICD Ab (middle panel), respectively, and GSK-3α and GSK-3β expressions were analyzed using anti-HA Ab (bottom panel). (B) Notch1 levels were increased by kinase-inactive forms of GSK-3α and GSK-3β. MEF cells were transfected with ΔEN1-myc and HA-tagged GSK-3α or GSK-3β kinase-inactive constructs as indicated. Notch1 and NICD expressions were determined using anti-myc (top panel) and anti-NICD Ab (middle panel), respectively, and kinase-inactive GSK-3α and GSK-3β expressions were determined using anti-HA Ab (bottom panel). (C, D) Notch1 levels were increased by GSK-3α/β (C) and GSK-3α siRNA (D). MEF cells were transfected with ΔEN1-myc and indicated concentrations of GSK-3α/β or GSK-3α siRNA. Notch1 and NICD levels were determined using anti-myc (top panel) or anti-NICD Ab (middle panel), respectively, and endogenous GSK-3α and GSK-3β levels were analyzed using anti-GSK-3α/β Ab (bottom panel). (E, F) Endogenous NICD levels were increased by GSK-3α/β (E) and GSK-3α siRNA (F). MEF cells were transfected with indicated concentrations of GSK-3α/β or GSK-3α siRNA. Endogenous NICD levels were determined using anti-NICD Ab (top panel), and endogenous GSK-3α and GSK-3β levels were analyzed using anti-GSK-3α/β Ab (bottom panel).

it is responsible for phosphorylating target proteins. To investigate whether Notch1 receptor and NICD levels are affected by GSK-3, we transfected MEF cells and 293 HEK cells with a constitutively active form of Notch1 tagged with myc (ΔEN1-myc), and then treated them with LiCl (a specific GSK-3 inhibitor). LiCl treatment increased Notch1 receptor and NICD levels in a concentration-dependent manner in MEF cells (top and middle panels, respectively, Fig. 1A) and in 293 HEK cells (data not shown). β-Catenin (a well-known target of GSK-3β) was used as a positive control to confirm LiCl concentration-dependent increases in its protein level (bottom panel in Fig. 1A). The results obtained suggested that the stabilities of Notch1 are regulated by GSK-3α/β. In addition, we examined whether LiCl affects NICD-dependent transcriptional activity and endogenous NICD levels, and we found that endogenous NICD levels were increased by LiCl (Fig. 1B). 293 HEK cells were transfected with either a CSL luciferase construct (which contains 4 CSL binding sites and responds to NICD) or with ΔEN1-myc construct, and both endogenous and exogenous NICD dependent transcriptional activities were found to be dramatically increased by LiCl in a dose-dependent manner (Figs. 1C and 1D, respectively). The stabilization of Notch1 by LiCl encouraged us to examine whether the mRNA levels of Notch1 target genes, such as, Hes1 are increased or not by LiCl. Accordingly, MEF cells were transfected with ΔEN1-myc and then treated with LiCl, and RT-PCR was performed on cDNA obtained from total RNA. As shown by Fig. 1E, the mRNA level of Hes1 was found to be increased by LiCl. In order to compare relative stabilities of Notch1 and NICD, we compared the half-lives of ΔEN1 and NICD in GSK-3β -/- fibroblasts. Treatment with cycloheximide (a protein synthesis inhibitor) showed that the half-life of NICD in GSK-3β -/- fibroblasts (8 h) was slightly longer than that of ΔEN1 (5 h) (Fig. 1F). These results indicated that GSK-3 does affect Notch1/NICD stability.

To confirm that the levels of Notch1 receptor and NICD are regulated by GSK-3α/β, we used HA tagged wild type and kinase inactive forms of the two GSK-3 isoforms. MEF cells were transfected with ΔEN1-myc together with increasing amounts of HA-tagged wild type GSK-3α or GSK-3β, and Notch1 receptor and NICD levels were found to be dramatically decreased by both (top and middle panels, respectively, Fig. 2A). On the other hand, the levels of Notch1 receptor and NICD were dramatically increased when GSK-3α and GSK-3β kinase inactive forms were expressed (top and middle panels, respectively, Fig. 2B). In order to prove that GSK-3α/β negatively regulates both Notch1 receptor and NICD levels, we knocked down the levels of either endogenous GSK-3α/β or GSK-3α by GSK-3α/β- or GSK-3α-specific siRNAs, respectively, and investigated the effects on Notch1/NICD. As shown in Figs. 2C and 2D, levels of exogenous Notch1 were increased by treatments with either GSK-3α/β- or GSK-3α-specific siRNA. In consistent with these exogenous data, endogenous NICD levels were also increased by either GSK-3α/β- or GSK-3α-specific siRNA as well (Figs. 2E and 2F, respectively). These results demonstrate

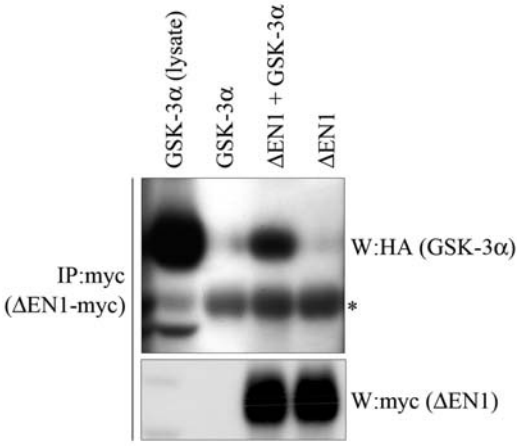

Fig. 3. Notch1 binds to GSK-3α *in vivo*. MEF cells were transfected with ΔEN1-myc and HA-tagged GSK-3α or ΔEN1-myc only. To examine Notch1 to GSK-3α binding, immunoprecipitation was performed using myc Ab, and immunoprecipitates were blotted using HA Ab (top panel). ΔEN1 expression was detected using myc Ab (bottom panel). The asterisk indicates a non-specific band.

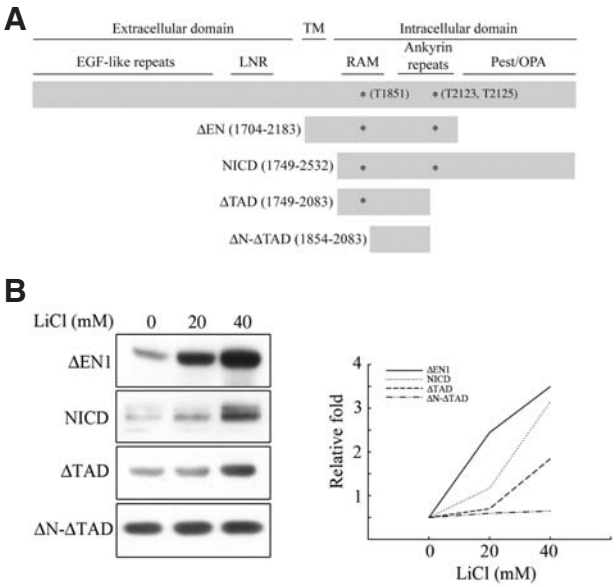

Fig. 4. Identification of the GSK-3 responding site in Notch1. (A) Schematic structure of Notch1. ΔEN1 contained three putative GSK-3β recognition sites (amino acids T1851, T2123, and T2125), ΔTAD contained only one putative recognition site (amino acid T1851), and ΔN-ΔTAD contained no recognition site. Each construct was myc-tagged. (B) Effects of LiCl on deleted Notch1 constructs. Constructs were transfected into MEF cells, which were then treated with LiCl. Notch1 expression was examined using anti-myc Ab. Protein expression levels were quantified (graph on right panel).

that GSK-3α/β negatively regulates both Notch1 receptor and NICD levels.

GSK-3α binds to Notch1 *in vivo*, and at least three Thr residues (Thr-1851, -2123, and -2125) of Notch1 are critical for its response to GSK-3α/β

To investigate whether GSK-3α binds Notch1 receptor, *in vivo* immunoprecipitation was performed in 293 HEK cells. Notch1 receptor was found to bind GSK-3α *in vivo* (Fig. 3), suggesting that Notch1 and NICD levels are regulated by the interaction between Notch1 and GSK-3α.

ΔEN1-myc has several putative GSK-3 recognition sites. The most likely of which are amino acid Thr-1851, Thr-2123, and Thr-2125; based on computerized analysis of protein motif scans. Therefore, we prepared deletion constructs to identify the domain of Notch1 targeted by GSK-3α/β (Fig. 4A). Levels of ΔEN1 and NICD containing three putative GSK-3 recognition sites were dramatically induced by increasing LiCl concentrations in MEF cells. In particular, ΔEN1 which contained the transmembrane domain was most affected by LiCl. Levels of ΔTAD, which contained one putative GSK-3 recognition site but no TMD, were also affected by LiCl, but this effect was weak compared with its effect on ΔEN1 and NICD containing all putative GSK-3 recognition sites. On the other hand, levels of ΔN-ΔTAD, which did not contain TMD or GSK-3 recognition sites, almost unaffected (Figs. 4B and 4C). These results suggest that Notch1, tethered to the membrane and containing GSK-3 recognition sites, has greatest affinity for GSK-3.

DISCUSSION

This report suggests for the first time that GSK-3α negatively regulates Notch1/NICD. Although it has been previously reported that Notch1 is a GSK-3β substrate, no relation between Notch1 and GSK-3α (a homologue of GSK-3β) has been previously reported. Recently, it was reported that nuclear level of β-catenin (a known GSK-3β target) can be regulated by GSK-3α as well, and that GSK-3α also inhibits β-catenin-directed Tcf/Lef-dependent transcription (Asuni et al., 2006). On the other hand, findings of studies on the effect of GSK-3β on the stability and/or transcriptional control of Notch are contradictory. One study reported that Notch2 activity is downregulated after its phosphorylation by GSK-3β (Espinosa et al., 2003), but another study demonstrated that the stability and transcriptional activity of Notch1 are increased by GSK-3 (Santiago et al., 2002). On the other hand, the present study shows that Notch1 can be regulated by both GSK-3β and GSK-3α. Furthermore, a previous report showed that LiCl (in therapeutic concentrations) reduces Aβ formation from APP, but does not affect NICD formation from Notch, although APP and Notch are proteolytic substrates of γ-secretase (Phiel et al., 2003). However, our results show that Notch1/NICD levels are dramatically affected by LiCl. These result differences may be attributable to the different LiCl concentrations applied or cell types. Furthermore, the present study provides first evidence of binding between Notch1 and GSK-3α, which suggests that Notch1 might be regulated via Notch1 to GSK-3α binding.

Although the GSK-3α and GSK-3β isoforms have highly homologous kinase domains, their C- and N-terminal sequences are dissimilar, which suggests they differ functionally. Furthermore, they have different chromosomal locations in the human genome [i.e., GSK-3α on 19q13.1-q13.2 and GSK-3β on 3q13.3-q21 (Shaw et al., 1998)], and have differential expression patterns in some tissues (Lau et al., 1999; Yao et al., 2002). In addition, several recent studies have reported they have different functions *in vivo*. For example, GSK-3α was not able to rescue GSK-3β-null mice (Hoeflich et al., 2000), and while EGFR-induced keratinocyte migration was found to be activated by GSK-3α, it was inhibited by GSK-3β (Koivisto et al., 2006). Furthermore, in Alzheimer's disease, the productions of $A\beta_{40}$ and $A\beta_{42}$ peptides from APP is attributed to GSK-3α and

not GSK-3β (Wilson et al., 2003). Thus, evidence demonstrates that the two isoforms of GSK-3 have different functions, but that their activation/deactivation mechanisms are identical, i.e., activation via phosphorylation at a threonine residue, and deactivation via phosphorylation at a serine residue by Akt. In addition, Wnt signaling and the stimulation of insulin-induced glycogen deposition are regulated by GSK-3 phosphorylation mechanism (Alessi et al., 1995). Recently, it was reported that GSK-3α and GSK-3β are functionally redundant in terms of Wnt/β-catenin signaling (Doble et al., 2007), and in a previous study, we postulated that GSK-3α and GSK-3β act similarly in terms of the control of Notch1 levels, much like their affinities for β-catenin as substrate (Hooper et al., 2006).

Notch2 receptor binds more efficiently to GSK-3β than NICD, but NICD is a better substrate for GSK-3β phosphorylation (Espinosa et al., 2003). Here, we found that Notch1/NICD levels can be down-regulated by GSK-3α/β. In particular, the effect of LiCl on NICD levels was less than on ΔEN1. It might be that Notch1 in plasma membrane reacts more efficiently than NICD in cytosol with GSK-3α/β. Moreover, because minimal ΔN-ΔTAD (N1854-2083), which contains neither TM nor a putative GSK-3 phosphorylation site, did not respond to LiCl, we suggest that the effective sites of Notch1 for GSK-3α/β are at Thr-1851, 2123, and 2125 in its cytoplasmic domain. LiCl increased Notch1 receptor and NICD levels, which subsequently were found to be correlated with the transcriptional regulation of NICD. Furthermore, we obtained similar results in different cell types. For example, much higher increases in NICD-dependent transcription were observed in 293 HEK cells (15-20 fold) than in MEF cells. Actually, levels of endogenous NICD were increased by LiCl, and we observed a similar pattern for exogenous Notch1, which supports the idea that GSK-3α/β regulates NICD-dependent transcription. Although we suggest that Notch1 and NICD are substrates of GSK-3α and GSK-3β, it remains to be determined whether the roles of GSK-3α and GSK-3β on Notch signaling can be differentiated.

In this study, we suggest that GSK-3α (like GSK-3β) can act as a negative regulator of Notch1/NICD. Given the importance of the various pathways impacted by Notch, it is evident that Notch must be exquisitely well regulated. Although the activations and regulations of GSK-3α and GSK-3β have not been well established, it becomes apparent that precise regulations of these proteins are required for the delicate modulation of Notch protein itself.

ACKNOWLEDGMENTS

This study was supported by a grant from the Basic Research Program of the Korean Science and Engineering Foundation (R01-2006-000-10487-0) (to K.K.), Republic of Korea.

REFERENCES

Asuni, A.A., Hooper, C., Reynolds, C.H., Lovestone, S., Anderton, B.H., and Killick, R. (2006). GSK-3alpha exhibits beta-catenin and tau directed kinase activities that are modulated by Wnt. Eur. J. Neurosci. *24*, 3387-3392.

Brou, C., Logeat, F., Gupta, N., Bessia, C., LeBail, O., Doedens, J. R., Cumano, A., Roux, P., Black, R.A., and Israel, A. (2000). A novel proteolytic cleavage involved in Notch signaling: the role of the disintegrin-metalloprotease TACE. Mol. Cell *5*, 207-216.

Cross, D.A., Alessi, D.R., Cohen, P., Andjelkovich, M., and Hemmings, B.A. (1995). Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature *378*, 785-789.

Doble, B.W., Patel, S., Wood, G.A., Kockeritz, L.K., and Woodgett, J.R. (2007). Functional redundancy of GSK-3alpha and GSK-3beta in Wnt/beta-catenin signaling shown by using an allelic series of embryonic stem cell lines. Dev. Cell *12*, 957-971.

Espinosa, L., Ingles-Esteve, J., Aguilera, C., and Bigas, A. (2003). Phosphorylation by glycogen synthase kinase-3 beta down-regulates Notch activity, a link for Notch and Wnt pathways. J. Biol. Chem. *278*, 32227-32235.

Foltz, D.R., Santiago, M.C., Berechid, B.E., and Nye, J.S. (2002). Glycogen synthase kinase-3beta modulates notch signaling and stability. Curr. Biol. *12*, 1006-1011.

Goto, H., Kawano, K., Kobayashi, I., Sakai, H., and Yanagisawa, S. (2002). Expression of cyclin D1 and GSK-3beta and their predictive value of prognosis in squamous cell carcinomas of the tongue. Oral Oncol. *38*, 549-556.

Grimes, C.A., and Jope, R.S. (2001). The multifaceted roles of glycogen synthase kinase 3beta in cellular signaling. Prog. Neurobiol. *65*, 391-426.

Hoeflich, K.P., Luo, J., Rubie, E.A., Tsao, M.S., Jin, O., and Woodgett, J.R. (2000). Requirement for glycogen synthase kinase-3beta in cell survival and NF-kappaB activation. Nature *406*, 86-90.

Hughes, K., Nikolakaki, E., Plyte, S.E., Totty, N.F., and Woodgett, J.R. (1993). Modulation of the glycogen synthase kinase-3 family by tyrosine phosphorylation. EMBO J. *12*, 803-808.

Iso, T., Kedes, L., and Hamamori, Y. (2003). HES and HERP families: multiple effectors of the Notch signaling pathway. J. Cell Physiol. *194*, 237-255.

Kim, H., Ki, H., Park, H.S., and Kim, K. (2005). Presenilin-1 D257A and D385A mutants fail to cleave Notch in their endoproteolyzed forms, but only presenilin-1 D385A mutant can restore its gamma-secretase activity with the compensatory overexpression of normal C-terminal fragment. J. Biol. Chem. *280*, 22462-22472.

Koivisto, L., Jiang, G., Hakkinen, L., Chan, B., and Larjava, H. (2006). HaCaT keratinocyte migration is dependent on epidermal growth factor receptor signaling and glycogen synthase kinase-3alpha. Exp. Cell Res. *312*, 2791-2805.

Kopan, R. (2002). Notch: a membrane-bound transcription factor. J. Cell Sci. *115*, 1095-1097.

Lau, K.F., Miller, C.C., Anderton, B.H., and Shaw, P.C. (1999). Expression analysis of glycogen synthase kinase-3 in human tissues. J. Pept. Res. *54*, 85-91.

Lee, H.C., Tsai, J.N., Liao, P.Y., Tsai, W.Y., Lin, K.Y., Chuang, C.C., Sun, C.K., Chang, W.C., and Tsai, H.J. (2007). Glycogen synthase kinase 3 alpha and 3 beta have distinct functions during cardiogenesis of zebrafish embryo. BMC Dev. Biol. *7*, 93.

Liao, X., Thrasher, J.B., Holzbeierlein, J., Stanley, S., and Li, B. (2004). Glycogen synthase kinase-3beta activity is required for androgen-stimulated gene expression in prostate cancer. Endocrinology *145*, 2941-2949.

Logeat, F., Bessia, C., Brou, C., LeBail, O., Jarriault, S., Seidah, N.G., and Israel, A. (1998). The Notch1 receptor is cleaved constitutively by a furin-like convertase. Proc. Natl. Acad. Sci. USA *95*, 8108-8112.

Phiel, C.J., Wilson, C.A., Lee, V.M., and Klein, P.S. (2003). GSK-3alpha regulates production of Alzheimer's disease amyloid-beta peptides. Nature *423*, 435-439.

Shaw, P.C., Davies, A.F., Lau, K.F., Garcia-Barcelo, M., Waye, M.M., Lovestone, S., Miller, C.C., and Anderton, B.H. (1998). Isolation and chromosomal mapping of human glycogen synthase kinase-3 alpha and -3 beta encoding genes. Genome *41*, 720-727.

Wang, Q.M., Fiol, C.J., DePaoli-Roach, A.A., and Roach, P.J. (1994). Glycogen synthase kinase-3 beta is a dual specificity kinase differentially regulated by tyrosine and serine/threonine phosphorylation. J. Biol. Chem. *269*, 14566-14574.

Woodgett, J.R. (1990). Molecular cloning and expression of glycogen synthase kinase-3/factor A. EMBO J. *9*, 2431-2438.

Yao, H.B., Shaw, P.C., Wong, C.C., and Wan, D.C. (2002). Expression of glycogen synthase kinase-3 isoforms in mouse tissues and their transcription in the brain. J. Chem. Neuroanat. *23*, 291-297.