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Involvement of GSK-3β in TWEAK-mediated NF-κB activation

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Abstract Glycogen synthase kinase-3β (GSK-3β) is a key component of several signaling pathways. We found that a short variant of 'TNF-like weak inducer of apoptosis' (shortTWEAK) formed a complex with GSK-3β in a yeast two-hybrid system. We demonstrate that shortTWEAK and GSK-3β colocalize in the nucleus of human neuroblastoma cells. We also show that TWEAK is internalized in different cell lines and that it translocates to the nucleus. This event causes the degradation of IBα, the nuclear translocation of both GSK-3β and p65, and the induction of NF-B-driven gene expression. We demonstrate that the induction of IL-8 expression by TWEAK can be counteracted by LiCl. Taken together, these data suggest that GSK-3β plays an important role in the signal transduction pathway between TWEAK and NF-B.

Keywords: TWEAK; GSK-3β; NF-κB

1. Introduction

Glycogen synthase kinase-3β (GSK-3β) phosphorylates and thereby regulates the function of many metabolic, signaling and structural proteins. Recently, it was reported that mice with inactivated GSK-3β die during embryonic development due to massive hepatocyte apoptosis, caused by a defect in the NF-κB pathway. This defect was independent of DNA-binding activity, suggesting the involvement of GSK-3β in the regulation of p65 transactivation [1]. The role of GSK-3β in the regulation of NF-κB activation was also demonstrated by other groups [2,3]. These data are in contradiction with the demonstration that overexpression of a constitutive active mutant of GSK-3β strongly inhibited NF-κB activation [4].

Recent studies have indicated that GSK-3β localization is dynamically regulated, but the mechanisms by which it is controlled remain unclear. Although GSK-3β is found predominantly in the cytoplasm of most cells, it has been found to enter the nucleus during S-phase of the cell cycle and during apoptosis [5–8]. Analysis of the amino acid sequence does not reveal the presence of any recognizable nuclear import or export sequences, but it was suggested that GSK-3β localization may be regulated indirectly through associations with binding proteins [9].

'TNF-like weak inducer of apoptosis' (TWEAK) is a novel member of the TNF superfamily [10]. The TNF family members, currently including at least 18 ligands and 28 receptors, have pivotal roles in host defense, inflammation, apoptosis, autoimmunity and organogenesis [11]. The extracellular sequence of TWEAK shows the greatest similarity to that of TNF [12]. It was suggested that TWEAK exists in two different forms: an 18 kDa processed form which can be secreted into the supernatant (sTWEAK) and a 30–35 kDa form with an intact transmembrane domain [10]. sTWEAK induced apoptosis and NF-κB activation in human cell lines [12]. It was also shown to act as a growth factor and as a potent inducer of angiogenesis [13].

Fn14 was cloned from a human vein endothelial cell (HUVEC) cDNA library as a receptor for TWEAK [14]. TWEAK mediates angiogenesis and endothelial cell proliferation by interacting with Fn14 [15]. Fn14 can associate with four distinct TRAF family members (1, 2, 3 and 5) and stimulate the NF-κB signaling pathway [16]. However, it was recently indicated that a second TWEAK receptor exists on RAW cells that is responsible for mediating TWEAK-induced differentiation of RAW cells into osteoclasts, since flow cytometry did not reveal the expression of Fn14 on RAW cells [17].

In this paper, we demonstrate the involvement of GSK-3β in the TWEAK/short variant of TWEAK (shortTWEAK) signaling pathway. We found that GSK-3β and shortTWEAK interacted in a yeast two-hybrid (Y2H) system. We observed the nuclear localization of shortTWEAK and the colocalization of GSK-3β and shortTWEAK in the nuclei of intact cells. Our results indicate that TWEAK can exert its pro-inflammatory effects without binding to its receptor Fn14 by internalizing the cells and translocating directly to the nucleus. This event causes the nuclear translocation of GSK-3β and p65 in these cells. TWEAK also activates the IL-8 promoter by direct

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Abbreviations: C.E., cytoplasmatic extract; N.E., nuclear extract; E-TWEAK, protein encoded by pECFP-C1/TWEAK; E-shortTWEAK, protein encoded by pECFP-C1/shortTWEAK; TWEAK-E, protein encoded by pECFP-N1/TWEAK; shortTWEAK-E, protein encoded by pECFP-N1/shortTWEAK; FL, full length IL-8 promoter construct; 98BP, 98 base pair IL-8 promoter construct; 3κB, construct containing 3 times IL-6 NF-κB responsive element

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activation of NF-κB and this activation can be abolished by LiCl, a known GSK-3β inhibitor.

2. Materials and methods

2.1. Cell culture

SHSY-5Y cells were grown in DMEM supplemented with 1× non-essential amino acids, 10% fetal calf serum (FCS), 2 mM L-glutamine and 50 μg/ml gentamycin. Jurkat T cells were grown in RPMI 1640 medium, supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine and penicillin (100 U/ml)-streptomycin (0.1 mg/ml). L929 cells were grown in DMEM supplemented with 5% newborn calf serum, 5% FCS, 2 mM L-glutamine and penicillin (100 U/ml)–streptomycin (0.1 mg/ml) (Life Technologies).

2.2. Western blotting

Following cell lysis, equal amounts of protein were separated by SDS–PAGE (Novex) and transferred to Immun-Blot PVDF membranes (Bio-Rad), which were then incubated with antibodies against TWEAK (S-20), p65 (C-20) or IκBα (C-21) (all three: Santa Cruz) or against GSK-3β (Transduction Laboratories).

2.3. Constructs

mRNA was isolated from SHSY-5Y cells using the Dynabeads mRNA Direct kit (Dynal), as recommended by the manufacturer. The entire coding sequence of hTWEAK (Accession No. AF055872) was RT-PCR generated from SHSY-5Y cell mRNA using the following primers: TWEAK sense (5′-ATGGCCGCCCGTCGGAGCCAGAGGCGGAGG-3′) and TWEAK antisense (5′-TCAGTGAACCTGGAAGAGTCCGAAGTAGGTGAGG-3′). The Titan One Tube RT-PCR Kit (Roche) was used according to the manufacturer's instructions. The pCR-XL-TOPO/TWEAK sequence was verified by sequence analysis.

An ATG was attached in frame to the shortTWEAK sequence picked up in the Y2H screen by PCR, thereby generating pCR-XL-TOPO/shortTWEAK (forward primer: ATGGACCAGGAGGAGCTGGTGGC and reverse primer: TCAGTGAACCTGGAAGAGTC).

The coding sequence of GSK-3β was isolated from the pCR-XL-TOPO/GSK-3β plasmid by PCR and subcloned into pECFP-C1 and pECFP-N1, thereby generating pECFP-C1/GSK-3β (encoding C-GSK-3β) and pECFP-N1/GSK-3β (encoding GSK-3β-C).

The TWEAK and shortTWEAK sequences were subcloned into pEYFP-C1 and pEYFP-N1. This resulted in the generation of pEYFP-C1/TWEAK (encoding E-TWEAK), pEYFP-C1/shortTWEAK (encoding E-shortTWEAK), pEYFP-N1/TWEAK (encoding TWEAK-E) and pEYFP-N1/shortTWEAK (encoding shortTWEAK-E).

2.4. Transfections

SHSY-5Y cells were plated at 140 000 cells/well on poly-L-lysine coated 8-well chamber slides (LAB-TEK). They were transfected with 0.5 μg DNA and 0.8 μl Lipofectamine 2000 (Invitrogen) per well according to the manufacturer's protocol. The living cells were visualized 16–24 h after transfection using a Zeiss Axiovert 100 M microscope with a Plan Apochromat 63×/0.14 oil objective.

2.5. Internalization experiments

Recombinant human TWEAK (sTWEAK), comprising the 155 C-terminal AA of TWEAK, was purchased from PeprotechEC. The protein was reconstituted in 10 mM Na phosphate buffer, pH 7.5, to a concentration of 1 mg/ml and further diluted with water to a concentration of 0.1 mg/ml. sTWEAK was diluted in fresh medium and added to the cells at 1 μg/million cells.

2.6. Nuclear and cytoplasmic extract preparations

Nuclear and cytoplasmic extracts were prepared using the NE-PER kit (Pierce) according to the manufacturer's protocol and the extracts were used for western blotting.

2.7. NF-κB-dependent reporter assay

L929 cells were stably transfected with the luciferase (luc) expression vector pGL3basic, in which the luc gene was regulated by either the human full length IL-8 promoter (FL) or a shorter 98 base pair construct (98BP) (free gifts of Dr. M. Mukaida [18]) or else a synthetic construct containing 3 times the IL-6 NF-κB responsive element (3κB), as described by Dr. W. Vanden Berghe et al. [19]. All reporter cells are also stably expressing β-galactosidase (β-gal) under the control of a house-keeping promoter (mouse 3-phosphoglycerate kinase) which was a kind gift of Dr. P. Soriano [20] (Fred Hutchinson Cancer Research Centre, Seattle, WA). Cells were seeded to confluency and treated with TNF (2000 IU/ml), sTWEAK and/or 40 mM LiCl for 6 h. Cells were lysed and both luc and β-gal contents were measured. By correcting luc for β-gal values, we can compensate for differences in protein concentration in the various samples.

3. Results

3.1. Identification of a new GSK-3β-binding partner

A human brain cDNA library was screened by the yeast two-hybrid assay method (Y2H) using wild-type GSK-3β as a bait. 14 clones encoded a polypeptide of 193 amino acid residues corresponding to the 193 C-terminal amino acids of TWEAK (AA 57–250). We named this new variant shortTWEAK for shorter form of TWEAK. Comparison of the 5′ end of shortTWEAK with the sequence of human chr17:72251296–7260089 (http://genome-test.cse.uscs.edu) revealed that the 5′ end of shortTWEAK corresponds to the start of exon 2 (BI 766766) and that shortTWEAK thus only consists of the extracellular part of TWEAK. All of the 14 clones missed an ATG but we decided to attach a start codon by means of PCR and thereby generate shortTWEAK (193 AA), since TWEAK exists as a naturally processed soluble cytokine (sTWEAK) (155 AA) [10] and Y2H results show that it is this part of TWEAK that is necessary for the interaction with GSK-3β.

3.2. Localization of TWEAK and shortTWEAK in human SHSY-5Y cell line

TWEAK was predicted to be a type II transmembrane protein without apparent signal sequence at the amino terminus [12]. To examine the localization of TWEAK and shortTWEAK, we cloned both cDNAs into the pEYFP-vector and used both N- and C-terminal EYFP-fusion proteins to exclude spatial interference of the EYFP-fusion protein.

Cells expressing E-TWEAK displayed a bright signal in either plasma or nuclear membrane (Fig. 1A), indicating that TWEAK can be located in both cellular membranes. These cells underwent a morphology change soon after the expression of E-TWEAK, but survived, suggestive of a differentiation-type event. TWEAK-E expressing cells show localization throughout the cytoplasm in vesicle-like structures (Fig. 1B), thereby excluding the differentiation of E-TWEAK expressing cells to be caused by the fusion of TWEAK and EYFP. The cytoplasmic localization of TWEAK-E is probably caused by cleavage at the stalk region of TWEAK (which has been previously reported) [10], resulting in a cleavage product which lacks the transmembrane part.

Cells expressing shortTWEAK-E showed a nuclear localization of shortTWEAK (Fig. 1C). Immunofluorescent analysis also indicated nuclear localization of TWEAK (results not shown). PSORT II analysis indicates that both TWEAK and shortTWEAK contain two possible nuclear localization signals (NLS) at AA 90–93 and AA 96–102 or AA 35–38 and AA 41–47, respectively. These NLS confirmed our findings that shortTWEAK can be a nuclear protein. In contrast, E-shortTWEAK expressing cells showed localization throughout the whole cytoplasm (see Fig. 1D) in vesicle-like structures possibly caused by shielding of these NLS by the EYFP-protein.

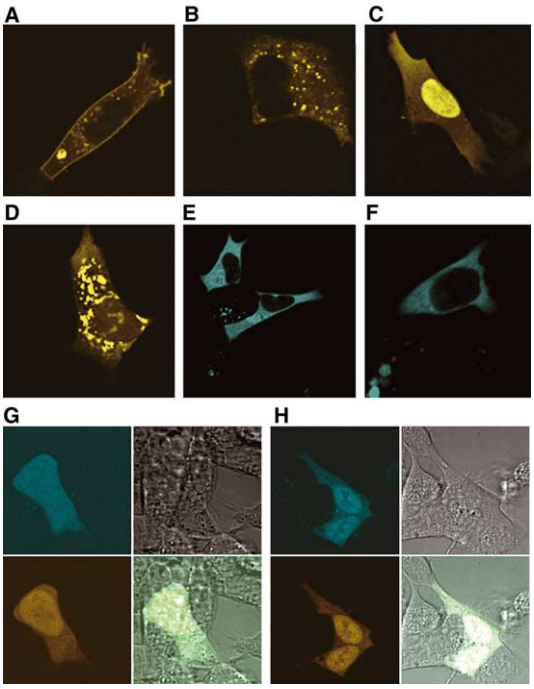

Fig. 1. Subcellular localization of TWEAK, shortTWEAK and GSK-3β. (A–F) Confocal images of SHSY-5Y cells transfected with vectors encoding E-TWEAK (A), TWEAK-E (B), shortTWEAK-E (C), E-shortTWEAK (D), C-GSK-3β (E) or GSK-3β-C (F). (G,H) Confocal analysis of SHSY-5Y cells co-expressing C-GSK-3β or GSK-3β-C and shortTWEAK-E: colocalization is shown as a merge of the two signals in the nuclei of the cells.

3.3. Colocalization of TWEAK and/or shortTWEAK with GSK-3β in human cells

Next, we studied the localization of GSK-3β and TWEAK versus shortTWEAK in living cells. Again, both N- and C-terminal GSK-3β-ECFP-fusion proteins were used. C-GSK-3β and GSK-3β-C expressing cells show localization throughout the cytoplasm and not at all in the nucleus (Fig. 1E and F). There was no colocalization when co-expressing GSK-3β, either fused to the N-terminus or C-terminus of ECFP, with E-TWEAK, TWEAK-E or E-shortTWEAK.

In contrast, colocalization in the nucleus was observed when we expressed GSK-3β, either fused to the N-terminus or C-terminus of ECFP, together with shortTWEAK-E (Fig. 1G and H). Cells expressing both proteins show apparent localization of GSK-3β, both nuclear and cytoplasmic. So, there is a clear translocation of GSK-3β to the nucleus when expressed simultaneously with shortTWEAK-E.

3.4. Internalization and subsequent translocation to the nucleus of sTWEAK in different cell lines

sTWEAK was added to different cell lines and we noticed that sTWEAK could be found in the nucleus of Jurkat, SHSY-5Y and L929 cells as soon as 10 min after the addition of sTWEAK to the cells. The signal reaches a maximum at about 25 min and stays the same until 60 min after the start of the incubation (Fig. 2).

Futaki reviewed that arginine-rich peptides possess the potential to translocate through the cell membrane and suggested the presence of an ubiquitous internalization mechanism for

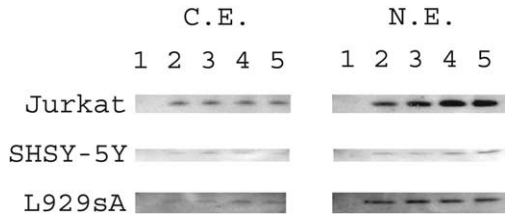

Fig. 2. Internalization and nuclear translocation of sTWEAK in different cell lines. Jurkat, SHSY-5Y and L929 cells were treated with 1 μg sTWEAK/million cells for an increasing amount of time (10, 25, 40, and 60 min: lanes 2–5) after which nuclear (N.E.) and cytoplasmic (C.E.) extracts were prepared. C.E. and N.E. were immunoblotted for TWEAK. Untreated cells served as negative control (lane 1).

arginine-rich peptides. The sequence of sTWEAK also shows multiple arginines located near the N-terminus (AA 1–10: MKGRKTRRAR). So, sTWEAK might use the same internalization mechanism as other membrane-permeable basic peptides, such as HIV-1 Tat, Antennapedia, human cFos and cJun peptides [21].

This result offers a new mode of action for TWEAK independent of its receptor Fn14 or any other receptor, since L929 and Jurkat cells were reported to lack Fn14 expression on the surface [22,23].

3.5. Effects of sTWEAK on the localization of GSK-3β and on the NF-κB pathway

GSK-3β is a cytosolic protein, but it is translocated into the nucleus when activated [24,25]. Since shortTWEAK colocalizes with GSK-3β in the nucleus of living cells, we wanted to see if the subcellular localization of GSK-3β changes after addition of sTWEAK to living cells. It was shown that sTWEAK increases NF-κB-specific DNA-binding activity [12] and since previous studies have shown that p65, and not p50, exerts transactivation potential, we examined whether there was also a translocation of p65 to the nucleus. As shown in Fig. 3A and B, as soon as 10 min after starting the incubation, we can observe an induction of both GSK-3β and p65 nuclear levels.

To examine if this effect was mediated by the canonical NF-κB activation pathway, we also analyzed the TWEAK-mediated IκBα degradation levels. We could see a time-dependent decrease in IκBα levels starting from 5 to 10 min, reaching a maximal decrease at 40 min. IκBα levels increased again after 60 min (Fig. 3C).

The rapid translocation of both GSK-3β and p65 to the nucleus, together with the effects seen on the IκBα levels, indicates that sTWEAK is a very potent stimulator for the translocation of GSK-3β and a strong activator of the canonical NF-κB pathway.

3.6. Effect of sTWEAK on NF-κB-specific DNA-binding activity and on the induction of the IL-8 promoter

Several groups reported that TWEAK induced NF-κB-specific DNA-binding activity and stimulated IL-8 secretion [10,12,26,27]. We examined if sTWEAK could induce NF-κB-dependent activation of the IL-8 promoter. Therefore, we used cell lines that express different IL-8 promoter-driven luciferase constructs: i.e., a full length IL-8 promoter construct (FL), or a 98 base pair fragment upstream of the start codon (containing C/EBP and NF-κB binding sites) (98BP) or else a construct containing 3NF-κB binding sites (3κB).

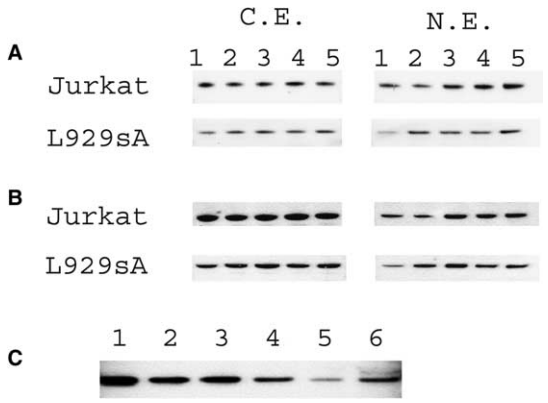

Fig. 3. Translocation of GSK-3β and p65 to the nucleus and degradation of IκBα in cells treated with sTWEAK. (A–B) Jurkat and L929 cells were treated with 1 μg sTWEAK/million cells for an increasing period of time (10, 25, 40, and 60 min: lanes 2–5) after which N.E. and C.E. extracts were prepared. C.E. and N.E. were immunoblotted for GSK-3β (A) and p65 (B). Untreated cells served as negative control (lane 1). (C) L929 cells were treated with 1 μg sTWEAK/million cells for an increasing amount of time (5, 10, 25, 40, and 60 min: lanes 2–6) after which total cell lysates were prepared. Cell lysates were immunoblotted for IκBα. Untreated cells served as negative control (lane 1).

NF-κB-dependent IL-8 promoter activity was induced in a concentration-dependent manner after stimulation of the cells with sTWEAK (Fig. 4A). The activity increased for all three cell lines using different concentrations in the range 0.25–20 nM. We used 20 nM sTWEAK in further experiments (see Fig. 4A). These data prove that IL-8 synthesis induced by TWEAK, as reported previously [10], is a direct consequence of the activation of the IL-8 promoter by TWEAK.

The stimulation of IL-8 promoter activity by sTWEAK results in a 2.5 to 3-fold induction in comparison with untreated cells and the relative inducibility remains comparable for the various cell lines (Fig. 4B). The fact that sTWEAK not only affects the complete IL-8 promoter but also the minimal NF-κB promoter indicates that the effect is mainly caused by the NF-κB activation. This is also supported by Harada et al. [28] who suggested that the TWEAK-induced secretion of IL-8 by HUVEC cells was abrogated by NF-κB inhibition. In comparison to sTWEAK, TNF causes a 6-fold induction for the full-length and 3κB promoter and a 13-fold induction for the 98 base pair promoter, respectively. sTWEAK is thus a less potent inducer of IL-8 promoter activity than TNF.

To investigate the possible involvement of GSK-3β in sTWEAK-induced activation of NF-κB-dependent gene expression, the effect of lithium chloride (a well-known GSK-3β inhibitor) [29] was examined. As shown in Fig. 4C, lithium chloride (40 mM) inhibited the effect of sTWEAK on the full-length (9%), 98 base pair (40%) and 3κB construct cells (53%). Similar results were obtained using kenpaullone and GSK-3β inhibitor I (both GSK-3β-specific inhibitors from Calbiochem) (results not shown).

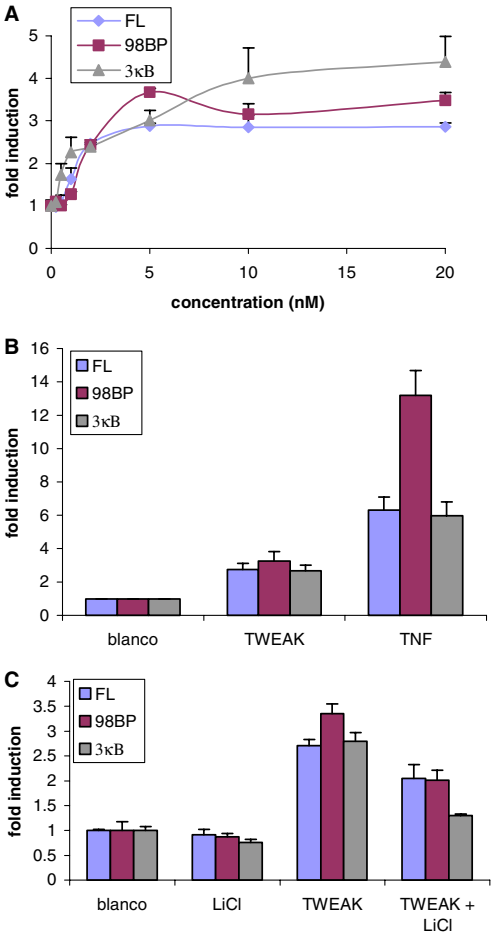

Fig. 4. Effect of sTWEAK, TNF and LiCl on different IL-8 promoter constructs. (A) Concentration dependent induction of NF-κB-dependent IL-8 promoter constructs. Stable cell lines expressing FL, 98BP and 3κB promoter constructs were incubated with different concentrations of sTWEAK (0.25; 0.5; 1; 2; 5; 10 and 20 nM). (B) Stimulation of different IL-8 promoter constructs with sTWEAK and TNF. Stable cell lines expressing FL, 98BP and 3κB promoter constructs were incubated with 20 nM sTWEAK or 2000 IU/ml TNF. (C) Influence of 40 mM LiCl on sTWEAK-stimulation. Stable cell lines expressing FL, 98BP and 3κB promoter constructs were incubated with 40 mM LiCl, 20 nM sTWEAK, or a combination. The results are expressed as fold induction compared to the non-treated condition. Data (means ± S.D.) are from at least three independent experiments.

4. Discussion

Members of the TNF family exist as transmembrane and soluble forms that elicit distinct, and sometimes opposing, effects on inflammatory and apoptotic mechanisms [10]. TWEAK was predicted to be a type II membrane-bound protein but it was suggested that it can also exert its apoptotic activity in the soluble form [12,30]. We identified a shorter form of TWEAK (shortTWEAK) to be an interaction partner for GSK-3β through a Y2H screen using GSK-3β as bait. This shortTWEAK protein consists of 40 AA more than sTWEAK.

The data presented above demonstrate that cells expressing full-length TWEAK show morphological changes but survived, suggesting a differentiation type of event. This is according to previous findings that TWEAK can induce morphology changes or differentiation of cells, depending on the cell type [10,17]. Transiently transfected shortTWEAK-E, however, showed nuclear localization. This is the first evidence for TWEAK or shortTWEAK to be found in the nucleus and this indicates that TWEAK and shortTWEAK may elicit distinct effects on signaling pathways.

The fact that GSK-3β and shortTWEAK colocalize in the nucleus indicates that the Y2H results also have a physiological

role in living cells. We did not succeed in confirming the direct interaction between TWEAK and GSK-3β through co-immunoprecipitation but there might be a need for a third interaction partner or a specific stimulus, necessary for interaction between both proteins.

GSK-3β localization was suggested to be regulated indirectly through associations with binding proteins [9]. GSK-3β phosphorylates and thereby regulates the nuclear export, DNA binding and transcriptional activity of a large range of substrates [31]. The finding that TWEAK can modulate the localization of GSK-3β thus raises the possibility that regulators of TWEAK can indirectly affect phosphorylation of various other substrates by GSK-3β.

We propose that shortTWEAK and sTWEAK, having the arginine-rich sequence in common, share the internalization mechanism, as suggested for other arginine-rich membrane permeable peptides [21]. This mechanism of internalization offers a new mode of action for TWEAK and an explanation for previous results showing a TWEAK response in cells without Fn14 expression (L929) [22]. We also observed a TWEAK response in Jurkat cells (not expressing Fn14), whereas others did not see any response [23].

The rapid degradation of IκBα and nuclear translocation of p65, after addition of sTWEAK to the cells, suggested that sTWEAK is a strong inducer of NF-κB activity. This is consistent with previous results indicating that RAW cells (cells without Fn14 expression) show rapid degradation of IκBα after addition of TWEAK [17]. The hypothesis of TWEAK internalization offers an explanation to these findings, without the involvement of a new TWEAK receptor.

Our results show that the translocation of sTWEAK, GSK-3β and p65 to the nucleus in L929 and Jurkat cells is reflected in at least one functional parameter, i.e., IL-8 promoter activity. The fact that sTWEAK not only affects the full length IL-8 promoter the minimal NF-κB promoter (3κB) indicates that the effect is mainly caused by NF-κB activation. This is also supported by Harada et al. [28] who suggested that the TWEAK-induced secretion of IL-8 by HUVEC cells was mediated by NF-κB activation.

An inhibitory effect of LiCl on the IL-8 promoter activation was observed during our experiments. We propose that the effect of GSK-3β on the NF-κB activation takes place at the level of the p65 transactivation, as suggested by Hoeflich et al. [1]. The different levels of inhibition seem to be related to the significance of the NF-κB responsive element on the promoter constructs: the larger the impact, the larger the inhibitory effect of LiCl.

Taken together, we have found that TWEAK has an effect on the NF-κB pathway and that GSK-3β participates in this pathway. Specifically, (1) shortTWEAK bound to GSK-3β in a Y2H assay, (2) shortTWEAK colocalized with GSK-3β in the nucleus of living cells, (3) TWEAK is internalized into living cells and translocates to the nucleus, (4) TWEAK stimulation of cells causes the nuclear translocation of GSK-3β and p65 and the degradation of IκBα and (5) the activation of the IL-8 promoter by TWEAK can be inhibited by LiCl, a commonly used GSK-3β inhibitor. This opens new perspectives on the GSK-3β signal transduction pathways and the modulation of inflammatory gene expression.

References

[1] Hoeflich, K.P., Luo, J., Rubie, E.A., Tsao, M.S., Jin, O. and Woodgett, J.R. (2000) Nature 406, 86–90.
[2] Schwabe, R.F. and Brenner, D.A. (2002) Am. J. Physiol. Gastrointest. Liver Physiol. 283, G204–G211.
[3] Bournat, J.C., Brown, A.M. and Soler, A.P. (2000) J. Neurosci. Res. 61, 21–32.
[4] Sanchez, J.F., Sniderhan, L.F., Williamson, A.L., Fan, S., Chakraborty-Sett, S. and Maggirwar, S.B. (2003) Mol. Cell Biol. 23, 4649–4662.
[5] Bijur, G.N., De Sarno, P. and Jope, R.S. (2000) J. Biol. Chem. 275, 7583–7590.
[6] Bijur, G.N. and Jope, R.S. (2001) J. Biol. Chem. 276, 37436–37442.
[7] Diehl, J.A., Cheng, M., Roussel, M.F. and Sherr, C.J. (1998) Genes Dev. 12, 3499–3511.
[8] Alt, J.R., Cleveland, J.L., Hannink, M. and Diehl, J.A. (2000) Genes Dev. 14, 3102–3114.
[9] Franca-Koh, J., Yeo, M., Fraser, E., Young, N. and Dale, T.C. (2002) J. Biol. Chem. 277, 43844–43848.
[10] Chicheportiche, Y., Bourdon, P.R., Xu, H., Hsu, Y.M., Scott, H., Hession, C., Garcia, I. and Browning, J.L. (1997) J. Biol. Chem. 272, 32401–32410.
[11] Liu, Y., Hong, X., Kappler, J., Jiang, L., Zhang, R., Xu, L., Pan, C.H., Martin, W.E., Murphy, R.C., Shu, H.B., Dai, S. and Zhang, G. (2003) Nature 423, 49–56.
[12] Marsters, S.A., Sheridan, J.P., Pitti, R.M., Brush, J., Goddard, A. and Ashkenazi, A. (1998) Curr. Biol. 8, 525–528.
[13] Lynch, C.N., Wang, Y.C., Lund, J.K., Chen, Y.W., Leal, J.A. and Wiley, S.R. (1999) J. Biol. Chem. 274, 8455–8459.
[14] Wiley, S.R., Cassiano, L., Lofton, T., Davis-Smith, T., Winkles, J.A., Lindner, V., Liu, H., Daniel, T.O., Smith, C.A. and Fanslow, W.C. (2001) Immunity 15, 837–846.
[15] Jakubowski, A., Browning, B., Lukashev, M., Sizing, I., Thompson, J.S., Benjamin, C.D., Hsu, Y.M., Ambrose, C., Zheng, T.S. and Burkly, L.C. (2002) J. Cell Sci. 115, 267–274.
[16] Brown, S.A., Richards, C.M., Hanscom, H.N., Feng, S.L. and Winkles, J.A. (2003) Biochem. J 371, 395–403.
[17] Polek, T.C., Talpaz, M., Darnay, B.G. and Spivak-Kroizman, T. (2003) J. Biol. Chem. 278, 32317–32323.
[18] Mukaida, N., Mahe, Y. and Matsushima, K. (1990) J. Biol. Chem. 265, 21128–21133.
[19] Vanden Berghe, W., Plaisance, S., Boone, E., De Bosscher, K., Schmitz, M.L., Fiers, W. and Haegeman, G. (1998) J. Biol. Chem. 273, 3285–3290.
[20] Soriano, P., Montgomery, C., Geske, R. and Bradley, A. (1991) Cell 64, 693–702.
[21] Futaki, S. (2002) Int. J. Pharm. 245, 1.
[22] Nakayama, M., Harada, N., Okumura, K. and Yagita, H. (2003) Biochem. Biophys. Res. Commun. 306, 819–825.
[23] Nakayama, M., Ishidoh, K., Kojima, Y., Harada, N., Kominami, E., Okumura, K. and Yagita, H. (2003) J. Immunol. 170, 341–348.
[24] Kim, L. and Kimmel, A.R. (2000) Curr. Opin. Genet. Dev. 10, 508–514.
[25] Ferkey, D.M. and Kimelman, D. (2000) Dev. Biol. 225, 471–479.
[26] Kaptein, A., Jansen, M., Dilaver, G., Kitson, J., Dash, L., Wang, E., Owen, M.J., Bodmer, J.L., Tschopp, J. and Farrow, S.N. (2000) FEBS Lett. 485, 135–141.
[27] Saas, P., Boucraut, J., Walker, P.R., Quiquerez, A.L., Billot, M., Desplat-Jego, S., Chicheportiche, Y. and Dietrich, P.Y. (2000) Glia 32, 102–107.
[28] Harada, N., Nakayama, M., Nakano, H., Fukuchi, Y., Yagita, H. and Okumura, K. (2002) Biochem. Biophys. Res. Commun. 299, 488–493.
[29] Klein, P.S. and Melton, D.A. (1996) Proc. Natl. Acad. Sci. USA 93, 8455–8459.
[30] Schneider, P. and Tschopp, J. (2000) Pharm. Acta Helv. 74, 281–286.
[31] Grimes, C.A. and Jope, R.S. (2001) Prog. Neurobiol. 65, 391–426.