

Oncoprotein p28^{GANK} binds to RelA and retains NF- κ B in the cytoplasm through nuclear export

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p28^{GANK} (also known as PSMD10, p28 and gankyrin) is an ankyrin repeat anti-apoptotic oncoprotein that is commonly overexpressed in hepatocellular carcinomas and increases the degradation of p53 and Rb. NF- κ B (nuclear factor- κ B) is known to be sequestered in the cytoplasm by I κ B (inhibitor of NF- κ B) proteins [1, 2], but much less is known about the cytoplasmic retention of NF- κ B by other cellular proteins. Here we show that p28^{GANK} inhibits NF- κ B activity. As a nuclear-cytoplasmic shuttling protein, p28^{GANK} directly binds to NF- κ B/RelA and exports RelA from nucleus through a chromosomal region maintenance-1 (CRM-1) dependent pathway, which results in the cytoplasmic retention of NF- κ B/RelA. We demonstrate that all the ankyrin repeats of p28^{GANK} are required for the interaction with RelA and that the N terminus of p28^{GANK}, which contains the nuclear export sequence (NES), is responsible for suppressing NF- κ B/RelA nuclear translocation. These results suggest that overexpression of p28^{GANK} prevents the nuclear localization and inhibits the activity of NF- κ B/RelA.

Keywords: p28^{GANK}, NF- κ B, CRM-1

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Introduction

The oncoprotein p28^{GANK}, containing five complete ankyrin repeats, is overexpressed in most hepatocellular carcinomas (HCCs) [3], and interacts with the CDK4 and S6b/TBP7 ATPases of the PA700/19S regulatory complex of the 26S proteasome, increasing the degradation of the retinoblastoma gene product [4, 5]. p28^{GANK} also directly binds to MDM2, which increases the p53-MDM2 association, inducing ubiquitylation and degradation of p53, and subsequently suppressing the pro-apoptotic signaling pathway mediated by p53 [6]. Our previous results have demonstrated that the significant downregulation of

p28^{GANK} in HCC cells by adenovirus-delivered siRNA targeting p28^{GANK} (Adsip28^{GANK}) inhibits cancer cell growth, and eventually results in apoptosis *in vitro* and *in vivo* [7]. However, the molecular mechanisms underlying the anti-apoptotic function of p28^{GANK} have not yet been fully elucidated.

NF- κ B transcription factors commonly function as homo- or heterodimers that are formed from the multigene family of RelA (p65), RelB, c-Rel, NF- κ B1 (p50/p105) and NF- κ B2 (p52/p100) [8, 9]. Members of the NF- κ B family are characterized by the presence of a Rel homology domain, which contains a nuclear localization sequence (NLS) and is involved in sequence-specific DNA binding, dimerization and interaction with the inhibitory I κ B proteins. Dimeric NF- κ B complexes are linked with specific responses to different stimuli and have differential effects on transcription. NF- κ B is predominantly found in the cytoplasm in an inactive form bound to the members of the I κ B family [10, 11]. NF- κ B has major roles in the inducible expression of a large number of genes that are involved in

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inflammation, host defense, cell survival and proliferation. In particular, the RelA NF- κ B subunit is associated with being a regulator of apoptosis [12, 13]. *relA* knockout mice die *in utero* as a result of massive liver apoptosis [14]. Whereas NF- κ B is most commonly involved in suppressing apoptosis, it can promote programmed cell death in response to certain death-inducing signals and in certain cell types [12, 15-17]. It was also shown to be indispensable for p53-dependent apoptosis [18]. Given that p28^{GANK} can negatively regulate p53-induced apoptosis, we initiated a series of experiments in this study that were designed to define the potential relationship between p28^{GANK} and NF- κ B activity.

We found that downregulation of p28^{GANK} by siRNA increases the basal NF- κ B transcriptional and DNA-binding activities, whereas overexpression of p28^{GANK} reduces them. We present a model in which p28^{GANK}, as a nuclear-cytoplasmic shuttling protein, associates directly with RelA to alter NF- κ B nuclear translocation. Structural studies reveal that this change in NF- κ B translocation is related to the nuclear export sequence (NES) of p28^{GANK}.

Materials and Methods

Cell culture and transfection

HepG2, Hep3B, 293T, 293A and HEK293 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, GIBCO), supplemented with 10% fetal bovine serum (GIBCO). HeLa cells were cultured in RPMI medium 1640 (GIBCO), supplemented with 10% newborn calf serum (GIBCO). The protocol used for the production and application of Adsip28^{GANK} or AdsiGFP has been previously described [7].

Reagents and plasmids

The transfection plasmids used were the pNF- κ B-Luc vector, which was obtained from Clontech, and the SV40-Renilla vector, which was obtained from Sigma. Anti-gankyrin, RelA, p50 and I κ B α antibodies were obtained from Santa Cruz, anti-Flag and β -actin antibodies were obtained from Sigma, anti-HA tag and Myc tag were obtained from Cell Signaling Technology. The whole cDNA sequence of p28^{GANK} containing HA-tag (from pCMV-HA-p28^{GANK}) was cloned into the pCDNA-3.1A-myc vector, which is used as the template for construction of a series of p28^{GANK} deletion mutants. The mutant deleting the N-terminus (amino acids 1-38) of p28^{GANK} (dN) was devoid of the HA-tag which was fused with the initial 38 amino acids of p28^{GANK}.

Luciferase reporter assays

Cells were infected with 15 MOI (multiplicity of infection) Adsip28^{GANK} or AdsiGFP 24 h before luciferase activity was measured. Cells were then transfected with 100 ng pNF- κ B-luc and 1 ng SV40-Renilla using jetPEI (Polyplus transfection), and stimulated with TNF- α (30 ng/ml, R&D). The effects of p28^{GANK} overexpression on NF- κ B activity were analyzed during co-transfection with the reporter plasmids and increasing amounts of the p28^{GANK} plasmids as above, and reporter activity was measured 24 h after transfection.

Electrophoretic mobility shift assay (EMSA)

The comparability of the various nuclear extracts was assessed in an EMSA using a Nuclear-Cytoplasmic Extract Kit (Pierce), according to the manufacturer's protocols. The probe containing the κ B DNA-binding consensus oligonucleotide (5'-AGTTGAGG-GGACTTTCCCAGGC-3') was radiolabeled with biotin (Sangon, China). Nuclear protein extracts (10 μ g) were incubated with the κ B-biotin-labeled probe and 1 μ g poly dI:dC (Promega) for 20 min at room temperature in a binding reaction buffer (10 mM Tris-HCl, pH 7.5, 10 mM KCl, 5 mM MgCl₂, 1 mM DTT, 5% glycerol). The DNA-protein complexes were resolved on 6% 0.5 \times TBE polyacrylamide mini-gels, according to the manufacturer's protocols (Pierce).

Immunofluorescence staining and confocal microscopy analysis

Cells were re-plated on chamber slides before transfection in the presence or absence of leptomycin B (LMB) (6 ng/ml, 2 h) from Merk. When cultured to 60-80% confluence, cells that were incubated with anti-RelA antibody conjugated to CY3 (Invitrogen) for immunofluorescence and confocal microscopic (Olympus) assay. Chromosomal DNA was visualized by staining with blue-fluorescent 4',6'-diamidino-2-phenylindole (DAPI, Molecular Probes).

Nuclear protein extraction, immunoblotting and immunoprecipitation analyses

The Nuclear Protein Extraction Kit (from Pierce) was used according to the manufacturer's recommendations for nuclear protein extraction. Extraction of cellular proteins was carried out according to the protocol described by Upstate Biotechnology and resolved on 8-18% Tris-tricine SDS-PAGE gradient gels. Immunoprecipitation was performed as previously described [27]. To analyze the interaction between endogenous p28^{GANK} and endogenous RelA, cells were lysed and immunoprecipitated with anti-gankyrin or anti-RelA antibody immobilized to the AminoLink[®] Plus Coupling Gel (Pierce), followed by immunoblotting.

Results

p28^{GANK} regulates NF- κ B activity

Our earlier studies have demonstrated that attenuation of p28^{GANK} in HCC induces apoptosis [7]. In an attempt to study whether the suppression of p28^{GANK} affects the activity of endogenous NF- κ B/RelA, three tumor cell lines (HepG2, Hep3B and HeLa) that overexpress p28^{GANK} were used. After infection with Adsip28^{GANK}, and compared with the control AdsiGFP (adenovirus-delivered siRNA targeting the green fluorescent protein), levels of p28^{GANK} protein were significantly reduced in these tumor cells (Figure 1A). After transfection with an NF- κ B-luciferase reporter gene, p28^{GANK} knockdown led to the obvious enhancement of the basal level NF- κ B transcriptional activity throughout the entire 72 h of the experiment. Tumor necrosis factor- α (TNF- α) stimulation caused further elevation of NF- κ B activity, even after p28^{GANK} knockdown (Figure 1B and data not shown). EMSA results also showed that, as expected, treatment with TNF- α increased the amount of nuclear NF- κ B bound to the DNA probe. Unlike AdsiGFP-treated

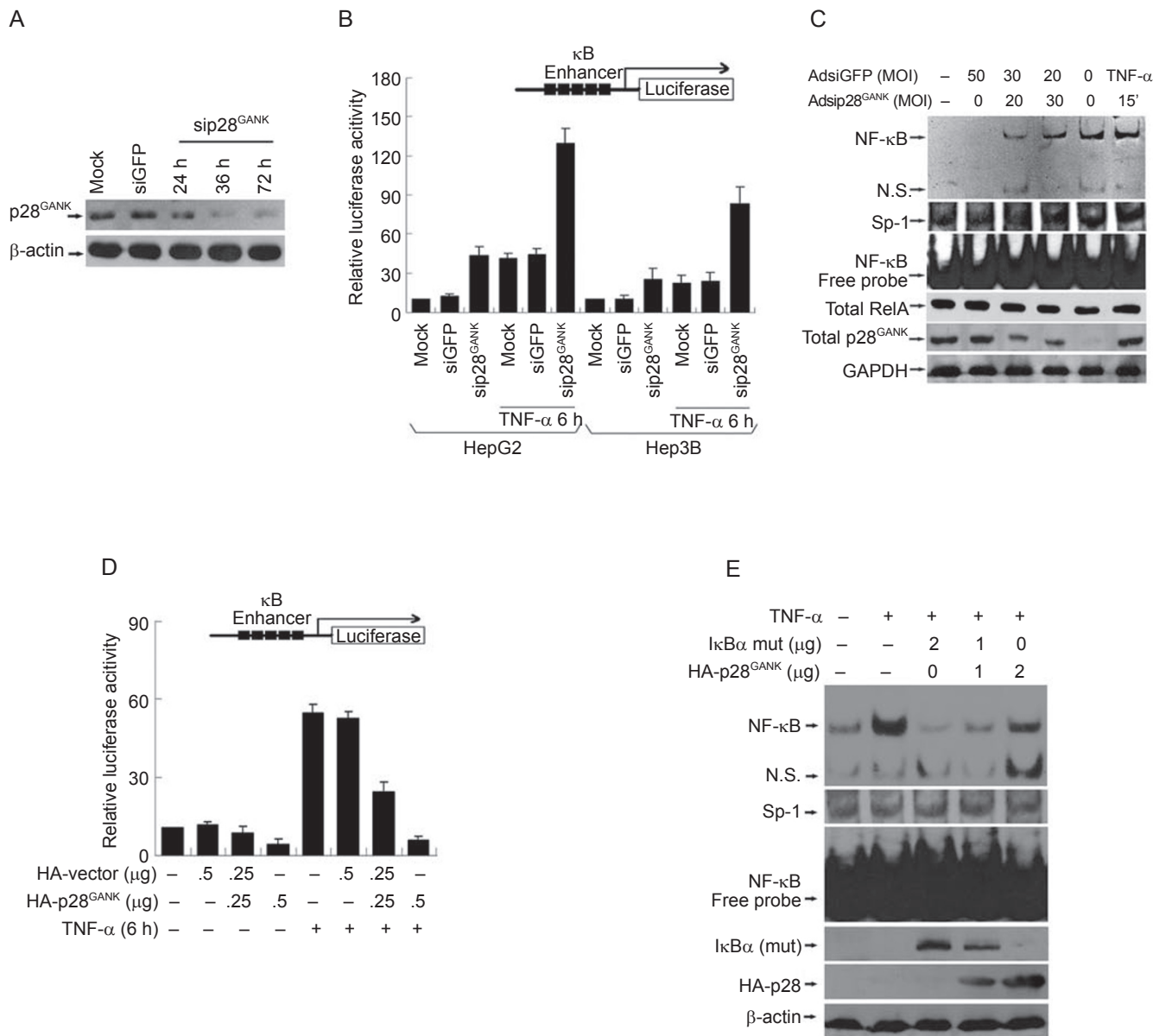


Figure 1 p28^{GANK} inhibits NF- κ B activity. **(A)** p28^{GANK} protein levels in HepG2 cells infected with Adsip28^{GANK} or AdsiGFP (negative control), respectively, were analyzed by western blot analysis using antibodies as indicated. **(B)** Decreased p28^{GANK} expression induced NF- κ B activity. HepG2 and Hep3B cells were infected with Adsip28^{GANK} or AdsiGFP. Cells were or were not stimulated with TNF- α (30 ng/ml). Here, luciferase reporter results only represent the 48-h time point after infection. The average value from three independent transfections (\pm S.D.) is shown. **(C)** p28^{GANK} knockdown increased NF- κ B DNA-binding activity. Nuclear extracts from the Adsip28^{GANK}- or AdsiGFP-infected HepG2 cells were assessed in an EMSA with a biotin-labeled NF- κ B consensus oligonucleotide (N.S., non-specific shift). Expression levels of the nuclear and total cell proteins were analyzed with the indicated antibodies. Comparability of the various nuclear extracts was verified by EMSA with a biotin-labeled Sp1 probe. **(D)** Effects on NF- κ B basal and induced activity by TNF- α stimulation. 293T cells were co-transfected with an NF- κ B luciferase reporter with HA-tagged p28^{GANK} or an empty vector. Cells were stimulated with TNF- α for 5 h before cells lysis, and then luciferase activity was determined. **(E)** p28^{GANK} overexpression decreased NF- κ B DNA-binding activity. 293T cells were co-transfected with RelA and Myc-tagged I κ B α mutant (mut), HA-tagged p28^{GANK} or an empty vector as indicated. Nuclear extracts were analyzed by EMSA. Expression levels of the nuclear and total extracts were analyzed by immunoblotting. The position of the NF- κ B-specific complex is indicated. Comparability of the various nuclear extracts was verified by EMSA with a biotin-labeled Sp1 probe.

or mock cells, depletion of p28^{GANK} in HepG2 tumor cells by Adsip28^{GANK} enhanced NF- κ B DNA-binding activity in a dose-dependent manner, but did not alter the binding of the constitutively expressed Sp1 transcription factor (Figure 1C). Next, we examined the effect of p28^{GANK} overexpression on NF- κ B activity. p28^{GANK} dose-dependently suppressed the basal or TNF- α induced NF- κ B activity in 293T cells (Figure 1D), but did not affect AP-1 activity (data not shown). EMSA analysis showed that p28^{GANK} also diminished NF- κ B DNA-binding activity, but did not affect Sp-1 DNA-binding activity (Figure 1E), indicating that p28^{GANK} itself suppresses both the transcriptional and DNA-binding activity of NF- κ B. To characterize the composition of the DNA-bound NF- κ B complexes in the presence of the suppression by p28^{GANK}, we performed supershift analysis with NF- κ B antibodies. These NF- κ B complexes were identified as heterodimers of RelA and p50, based on the ability of anti-RelA or anti-p50 to recognize the complexes (data not shown). Taken together, these results indicate that p28^{GANK} can regulate NF- κ B transcriptional and DNA-binding activities.

p28^{GANK} alters NF- κ B nuclear translocation

As p28^{GANK} suppression did not affect the amount of total RelA (Figure 1C), we next examined whether p28^{GANK} affects NF- κ B localization. First, we monitored the intracellular trafficking of endogenous RelA by immunostaining. As shown, constitutive RelA was diffusely present in the cytoplasm and nucleus in HeLa cells (Figure 2A). RelA increasingly translocated into the nucleus after p28^{GANK} knockdown, whereas no such change was noted in AdsiGFP-treated cells. When 293A cells were transfected with the plasmid expressing RelA, RelA accumulated in the nucleus (Figure 2B, top). However, after co-transfection with the p28^{GANK} plasmid, RelA was sequestered in the cytoplasm (Figure 2B, bottom). Consistent with this observation, overexpressing p28^{GANK} dose-dependently decreased the amount of nuclear RelA in 293T cells (Figure 2C). These results raise the possibility that p28^{GANK}, like the I κ B α protein, can enter the nucleus and displace NF- κ B from its DNA-binding sites, and then transport NF- κ B back to the cytoplasm, thereby carrying out a post-induction repression of NF- κ B function. Those results further support our conclusion that p28^{GANK} is a regulator of NF- κ B activity.

p28^{GANK} interacts directly with RelA and accelerates nuclear export of RelA through a chromosomal region maintenance-1 (CRM1)-dependent pathway

Because no complex formation between p28^{GANK} and I κ B α was detected by the immunoprecipitation assay (data not shown), we suspected that p28^{GANK} might interact directly with NF- κ B/RelA. Confocal microscopy analysis

of 293A cells revealed that RelA is sequestered in the cytoplasm and colocalized with GFP-p28^{GANK} (Figure 3A, top). In the presence of LMB, a nuclear export inhibitor that disrupts the interaction between CRM1/exportin 1 and the nuclear export signal [19], both GFP-p28^{GANK} and RelA colocalized mainly in the nucleus (Figure 3A, bottom). These observations illustrate that p28^{GANK}, acting as a nuclear-cytoplasmic shuttling protein, forms a complex with NF- κ B and promotes NF- κ B export from the nucleus to the cytoplasm through a CRM-1-dependent pathway.

Furthermore, co-immunoprecipitation of Flag-tagged RelA and Myc-tagged p28^{GANK} showed the physical interactions between these two proteins in HEK293 cells (Figure 3B). The association between endogenous p28^{GANK} and RelA was also observed in HepG2 cells (Figure 3C). Taken together, these data demonstrate that p28^{GANK} interacts directly with RelA *in vivo*.

Molecular mechanisms underlying p28^{GANK}-mediated nuclear export of RelA

It has been confirmed that the I κ B α protein retains NF- κ B in the cytoplasm by masking one of the two NLSs in NF- κ B dimers [20, 21]. To determine whether the NLS of RelA is required for p28^{GANK} binding, we constructed a series of mutants of RelA (Figure 4A) and carried out binding assays *in vivo*. Analysis of various mutant RelA proteins showed that the p28^{GANK}-interacting domain resides in residues 1-296 of RelA (Figure 4B), suggesting that the NLS of RelA is not required for the interaction. Interestingly, no interaction between p28^{GANK} and the mutant containing the N-terminal 312 residues of RelA was observed. The reason for this difference is not known and was not investigated further in this study.

Recent studies have indicated that an NES is located at the N terminus of the I κ B α protein, which functions to export the NF- κ B from the nucleus to the cytoplasm [22, 23]. p28^{GANK} consists of five ankyrin repeats that are flanked by two tails (Figure 4C), and actively shuttles between the nucleus and cytoplasm (Figure 3A). We constructed various mutants of p28^{GANK} by deleting a tail sequence or each of the ankyrin repeats to identify where the NES is located and which segment is required for the interaction of p28^{GANK} with RelA. As can be seen, only the mutant p28^{GANK} depleted of the N-terminal tail domain (amino acids 1-38) co-immunoprecipitated with RelA (Figure 4D), suggesting that all five ankyrin repeats and the C-terminal tail domain (amino acids 39-226) are required for p28^{GANK} binding to RelA. The N-terminal deletion mutant (dN) runs lower on the gel than all other mutants, because dN was not co-fused with HA-tag while others were during their construction.

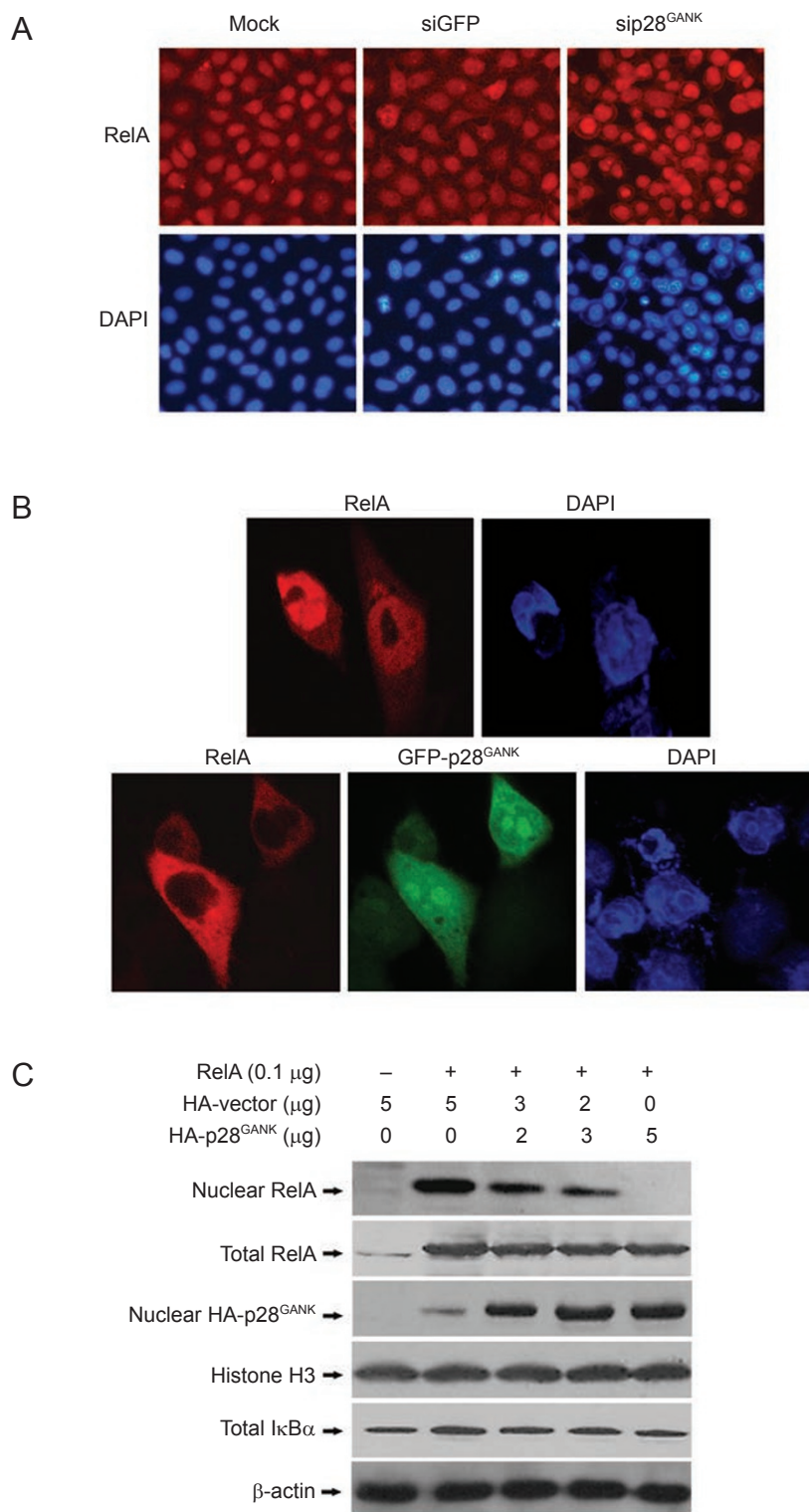


Figure 2 p28^{GANK} affects RelA/NF-κB nuclear translocation. **(A)** Increased RelA in the nucleus induced by p28^{GANK} depletion. Images of endogenous RelA localization were analyzed by immunostaining the mock or infected HeLa cells with AdsiGFP or Adsi p28^{GANK}. **(B)** Decreased RelA in the nucleus induced by increased p28^{GANK} expression. 293A cells were transfected with a RelA plasmid or in combination with GFP-p28^{GANK} and were analyzed by immunostaining. **(C)** Decreased nuclear RelA induced by p28^{GANK}. 293T cells were co-transfected with RelA and HA-tagged p28^{GANK} or an empty vector. The nuclear extracts and whole lysates were immunoblotted with the indicated antibodies.

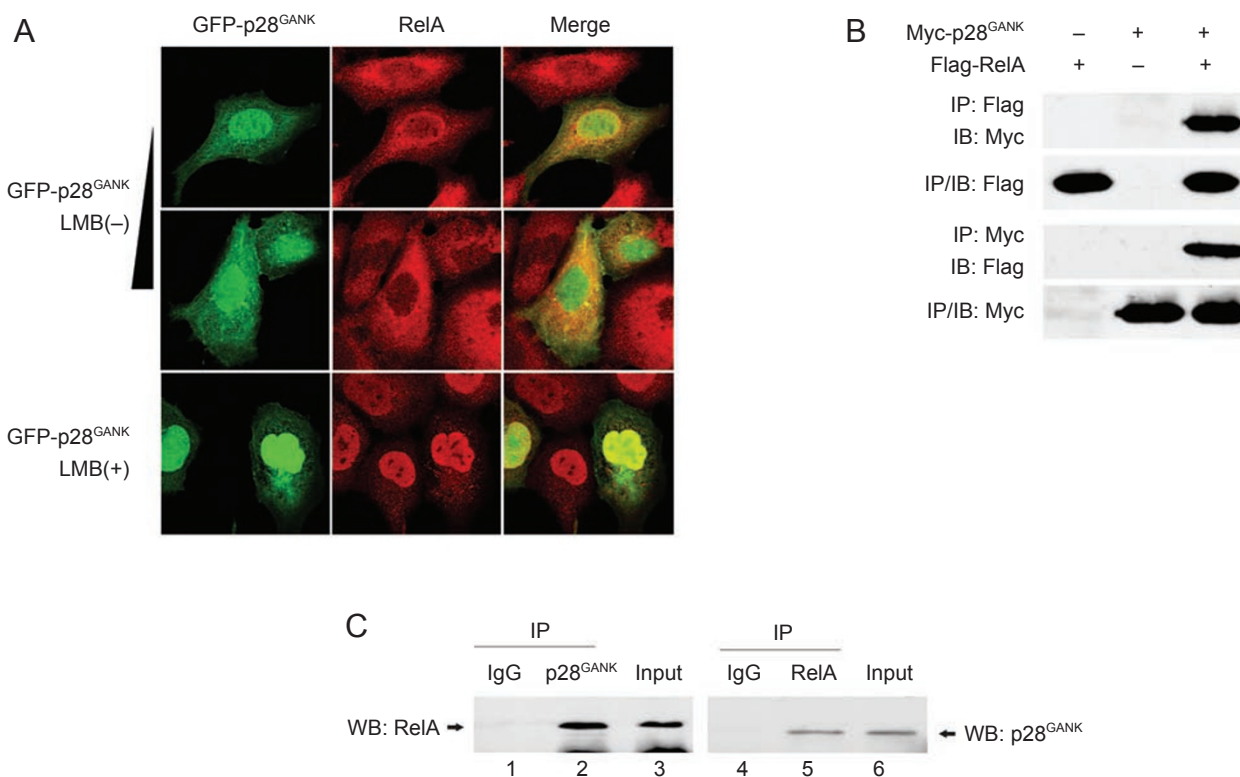


Figure 3 p28^{GANK} interacts directly with RelA. **(A)** p28^{GANK} colocalized with RelA in the cell. 293A cells were transfected with RelA plasmid or in combination with 1 μg/well (top panel) or 2 μg/well (middle panel) GFP-p28^{GANK}. They were then treated with or without LMB and were analyzed by confocal microscopy after staining with anti-RelA antibody, secondary antibody and DAPI. Green, red and blue signals represent the localization of p28^{GANK}, RelA and the nucleus. Yellow signals (merge) indicate colocalization of p28^{GANK} and RelA. **(B)** Interaction of overexpressed Myc-tagged p28^{GANK} with Flag-tagged RelA *in vivo*. HEK293 cells were co-transfected as indicated, and immunoprecipitated using Flag or Myc-tagged antibodies from cells. Immunoprecipitates (IPs) were immunoblotted for Myc-tagged p28^{GANK} or Flag-tagged RelA. **(C)** Interaction of endogenous p28^{GANK} with RelA *in vivo*. HepG2 cells were immunoprecipitated using p28^{GANK} (lane 2) or RelA (lane 5) antibodies, and anti-mouse IgG as a negative control (lanes 1 and 4). IPs were immunoblotted for endogenous RelA (lanes 1 and 2) or for endogenous p28^{GANK} (lanes 4 and 5) and whole-cell extracts for p28^{GANK} or RelA as indicated.

Analysis of p28^{GANK} structures required for NF-κB nuclear export

In general, IκBα contains leucine-rich NES transporting NF-κB back to the cytoplasm [24, 25]. To confirm whether p28^{GANK} contains NES that contributes to its translocation, we transfected the p28^{GANK} mutants and observed their distribution by immunostaining in 293T cells. Only the mutant with the N-terminal tail deleted was present in the nucleus (Figure 5A), indicating that p28^{GANK} contains a putative NES at its N terminus that mediates its shuttling out of the nucleus.

We next examined the ability of mutant p28^{GANK} proteins to export RelA to the cytoplasm. Overexpressed RelA in 293A cells was predominantly localized in the nucleus. Co-expression with either wild-type p28^{GANK} or IκBα promoted RelA to re-localize in the cytoplasm, but all the

mutant p28^{GANK} proteins failed to do so (Figure 5B and data not shown). The failure of p28^{GANK} mutants to retain RelA in the cytoplasm could arise from the removal of NES in mutant p28^{GANK} or from defects in association with RelA. These findings suggest that p28^{GANK} accelerates nuclear export of RelA, which requires the N-terminal domain of p28^{GANK}.

Discussion

In our study, we found that the oncoprotein p28^{GANK} inhibits NF-κB transcriptional and DNA-binding activity. Our data further confirm that p28^{GANK} is a nuclear-cytoplasmic shuttling protein that directly interacts with NF-κB and accelerates NF-κB export from the nucleus.

The prevailing view is that NF-κB proteins, in complex

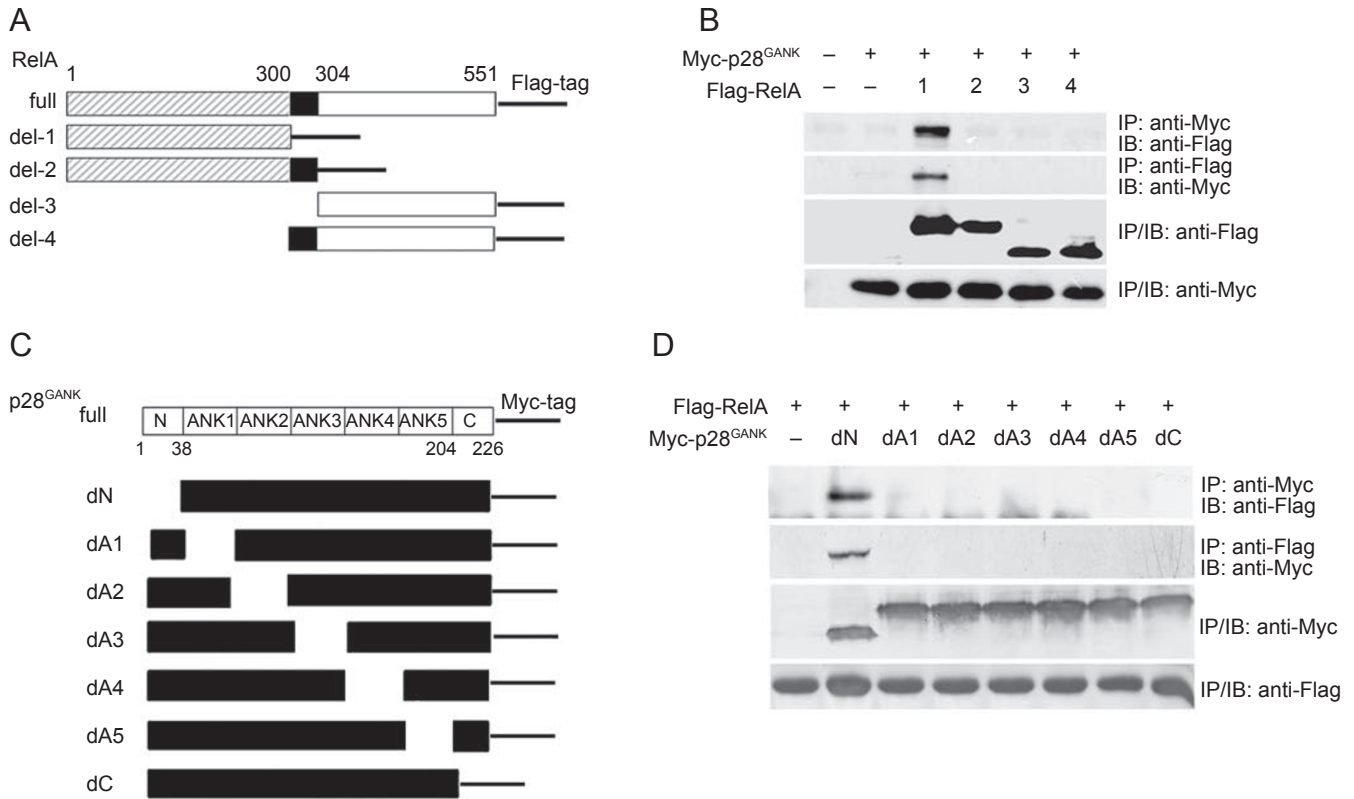


Figure 4 Molecular mechanisms of p28^{GANK} alter RelA nuclear translocation. **(A)** Schematic representation of RelA (full) and its mutants, all fused with Flag-tag. Black boxes, nuclear localization signal (NLS); shaded boxes, Rel homology domain. **(B)** Interaction of overexpressed Myc-tagged p28^{GANK} with Flag-tagged RelA mutants *in vivo*. HEK293 cells co-transfected with myc-tagged p28^{GANK} and Flag-tagged deletion mutants of RelA (del-1 to del-4) were immunoprecipitated and immunoblotted using Myc or/and Flag antibody. **(C)** Schematic representation of p28^{GANK} and its mutants with each of the ankyrin repeats (dA1 to dA5), the N terminus (dN) or the C terminus (dC) deleted, all fused with Myc-tag. **(D)** Interaction of overexpressed Flag-tagged RelA with Myc-tagged p28^{GANK} mutants *in vivo*. HEK293 cells co-transfected with Flag-tagged RelA and Myc-tagged deletion mutants of p28^{GANK} were immunoprecipitated and immunoblotted using Myc or/and Flag antibody.

with IκB family molecules, are sequestered in the cytoplasm in an inactive state. After activation by a large number of inducers, the IκB proteins undergo stimuli-coupled phosphorylation, ubiquitylation and proteasome-mediated degradation, which result in the liberation of the NF-κB dimers followed by the nuclear translocation of NF-κB. However, recent studies have indicated that IκBα, IκBβ and IκBε are not essential for the cytoplasmic sequestration of NF-κB and that there are other cellular factors that retain NF-κB in the cytoplasm, which are unresponsive to stimuli [26]. In our study, we found that the p28^{GANK} protein associates with NF-κB and retains it in the cytoplasm, even in the absence of IκB proteins (Chen and Li, in submission). Fujita *et al.* have also found another oncoprotein, hepatoma subtracted-cDNA library clone one (HSCO) [27], that binds to NF-κB and accelerates its export from the nucleus independent of IκBα. Together with these data,

our observations therefore demonstrate that there are indeed cellular proteins other than the known IκB family molecules that associate with NF-κB and regulate its cytoplasmic retention under basal conditions.

Traditionally it was believed that the inactive NF-κB: IκBα complex continuously shuttles in and out of the nucleus because IκBα masks one of the two nuclear localization signals (NLSs) in an NF-κB dimer and has an NES at its N terminus to achieve a dynamic balance of continuous movement between the nucleus and cytoplasm [28, 29]. Here, we found that p28^{GANK} can also act as a nuclear-cytoplasmic shuttling protein to export NF-κB from the nucleus, and that all ankyrin repeats of p28^{GANK} are indispensable for the interaction with NF-κB (Figures 4D and 5B). We constructed various RelA mutants and p28^{GANK} mutants to determine their interaction mechanisms. Although the NLS of RelA seems not to be involved in the binding, we cannot

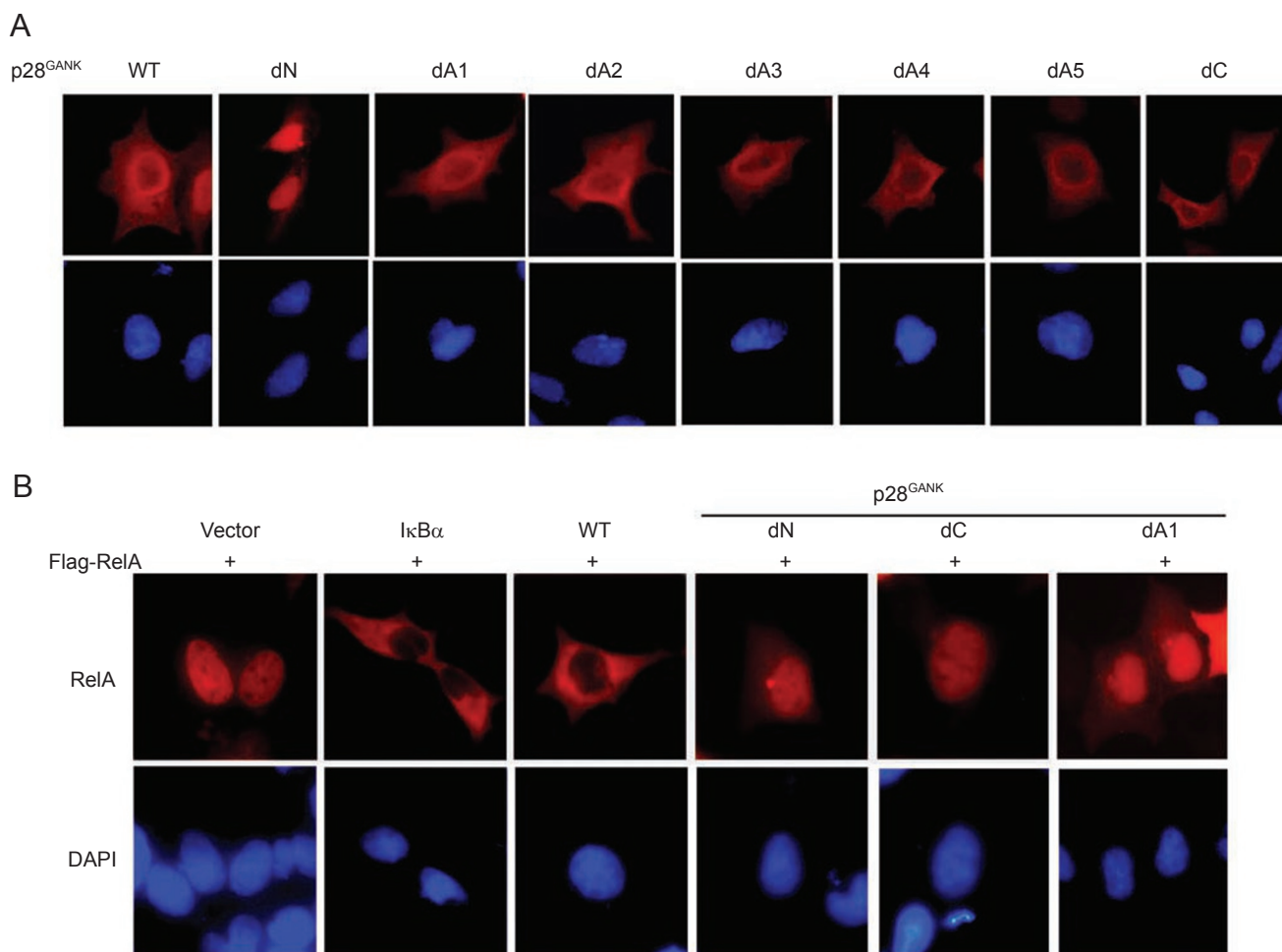


Figure 5 Structural analysis of the p28^{GANK} required for NF-κB nuclear transport. **(A)** Localization of p28^{GANK} and its deletion mutants in cells. HEK293 cells were transfected with Myc-tagged p28^{GANK} or deletion mutants. Red signals represent the localization of p28^{GANK} and the deletion mutants, and blue signals represent the localization of the nuclei. **(B)** Localization of RelA is affected by overexpression of p28^{GANK} or its depletion mutants. HEK293 cells were co-transfected with Flag-tagged RelA and Myc-tagged IκBα, Myc-tagged p28^{GANK}, or Myc-tagged RelA deletion mutants. Red signals represent the localization of RelA, and blue signals represent the localization of the nuclei.

rule out the possibility of p28^{GANK} masking this domain to maintain NF-κB inactivity. Next, we identified the potential location of the NES of p28^{GANK} as being at its N terminus (amino acids 1-38), which is responsible for the export of both p28^{GANK} itself and the NF-κB:p28^{GANK} complex out of the nucleus. Interestingly, our data showed that although the effects of p28^{GANK} on NF-κB DNA-binding activity and nuclear export are no stronger than that of IκBα, p28^{GANK} inhibits NF-κB transcriptional activity more significantly than IκBα. In addition to the cytoplasmic regulation of NF-κB, post-translational modifications of the NF-κB subunits and the histones that surround the NF-κB target genes have a key role in regulating transcription [30, 31]. As additional studies in our laboratory have revealed that

p28^{GANK} can directly decrease NF-κB acetylation, it is possible that there are other modifications of NF-κB, caused by p28^{GANK} along with some unknown repressors, that decrease NF-κB transcriptional activity.

Active NF-κB participates in the control of various target genes, including chemokines, immune receptors, adhesion molecules, stress response genes, regulators of apoptosis, transcription factors, growth factors, enzymes and cell-cycle regulators [17, 32]. RelA can be thought of as an anti-apoptotic or pro-apoptotic factor depending on the stimuli type and cell background [12, 16, 33-35]. Investigation of the expression of both NF-κB anti-apoptotic and pro-apoptotic target genes will help us to assess how p28^{GANK} affects RelA apoptotic function.

Hepatocellular carcinoma is one of the most common cancers worldwide and develops frequently in the context of chronic hepatitis, characterized by liver inflammation and hepatocyte apoptosis [36, 37]. NF- κ B is constitutively activated in many tumors and is thought to provide a crucial survival signal that assists cancer cells to escape apoptosis. However, other studies have revealed an anti-tumor function for NF- κ B in HCC [38]. Partial inhibition of NF- κ B led to liver cancer following chemically induced liver damage [39]. Overexpression of the oncoprotein p28^{GANK} in HCC acts as a small versatile cell-cycle regulator that regulates the activities of Rb and p53 [3, 6]. Our study suggests that the regulation of NF- κ B by p28^{GANK} might have critical roles in a variety of physiological and pathological processes. Further details about the molecular mechanisms underlying NF- κ B activity regulated by p28^{GANK} will contribute to an understanding of the regulation of NF- κ B pathways, which will provide a platform for developing specific therapeutics for diverse diseases.

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