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A novel zinc finger protein, ZZaPK, interacts with ZAK and stimulates the ZAK-expressing cells re-entering the cell cycle☆

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Abstract

ZAK has been implicated in cell cycle arrest regulation through its function on decreasing cyclin E expression. To explore the mechanistic basis for this regulation, the yeast two-hybrid system was used with a novel Krüppel-type C2H2 zinc finger member cloned. This cloned cDNA encodes a novel protein with Krüppel-type zinc fingers designed as ZZaPK (zinc finger and ZAK associated protein with KRAB domain) and is widely expressed. ZZaPK, when it is expressed in cells, is growth promoted and might lead to increasing E2F expression and induce cyclin E/CDK2 activity, which counteracts the ZAK function. The model proposed here is that ZAK might play a role as an upstream signal to suppress the ZZaPK function and decrease E2F expression.

Keywords: Leucine-zipper (LZ) and sterile-alpha motif (SAM) kinase (ZAK); Mixed lineage kinase (MLK); Zinc finger and ZAK associated protein with KRAB domain (ZZaPK); Cell cycle

In a previous study, a novel gene that encodes a serine/threonine kinase, designed ZAK for leucine-zipper (LZ) and sterile-alpha motif (SAM) kinase, was cloned [1]. The ZAK expression in mammalian cells leads specifically to JNK/SAPK pathway activation and NF-κB transcription factor. ZAK belongs to the mixed lineage kinase (MLK) family [2], comprising a group of highly related serine/threonine kinases that function as MAP3K [3–6]. ZAK expression, preferentially activating the JNK/SAPK pathway by MKK7 stimulation. An initial study indicated that one of the functions of ZAK is to arrest cell proliferation by causing G_2 arrest in the cell cycle [7]. The mechanism of this cell cycle arrest is due to ZAK activity that exerts its effect on decreasing the cyclin E expression levels.

Catalytic activity in a given CDK subunit depends on its correct post-translational modification and on its association with the appropriate cyclin [8–11]. Thus, phase-specific fluctuations in the abundance of various cyclins contribute one of the mechanisms that can regulate CDK activation and phase timing in the cell cycle. Cyclin E, a regulator subunit of cyclin-dependent kinase 2(CDK2), is a key regulator for mammalian cells to enter the cell cycle [12–14]. Cyclin E is one of the replication proteins that are regulated by the E2F transcription factor [15–17].

The E2F family of transcription factors is essential for the timely activation of genes involved in DNA replication and cell cycle control, exerting both positive and negative effects on gene expression. E2F activity is controlled in part by interactions with the pocket protein, pRB family [18,19]. This interaction represses the E2F-dependent gene expression. The ability of pRB to bind to E2F is regulated by its cell-cycle-dependent phosphorylation. As the cells progress through the cell cycle, pRB is hyperphosphorylated and causes the cell to release E2F. The resultant activation of E2F-responsive genes seems to be sufficient to commit the cells to initiate DNA replication [15].

Because ZAK expression causes G_2 arrest in the cell cycle and ZAK activity is necessary for cell cycle checkpoint regulation in cells. ZAK is abundantly

☆ *Abbreviations:* ZAK, leucine-zipper and sterile-alpha motif kinase; MLK, mixed lineage kinase; ZZaPK, zinc finger and ZAK associated protein with KRAB domain.

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expressed in human heart tissue. It is reasonable to search for ZAK-interacting proteins that are expressed in the human heart tissue. Here a novel zinc finger and ZAK-interacting protein with a KRAB domain, designated ZZaPK, was cloned. ZZaPK belongs to a known family of the KRAB family of transcriptional factors. Studies indicate that the ZZaPK protein can physically interact with ZAK. ZZaPK may contribute to E2F-mediated transcription activation and stimulate ZAK-expressing cells to re-enter the cell cycle.

Materials and methods

Two-hybrid screen and screening the plancenta cDNA library. The two-hybrid screen was performed as described in the user manual (Clontech). Briefly, the full-length ZAK was cloned to the pGBKT7 vector as the bait construct. This vector encoding GAL4 DNA-binding domain-ZAK fusion (DNA-BD/ZAK) was transformed into AH109 yeast. This pGBKT7-ZAK yeast was mated with Y187 yeast containing a pretransformed MATCHMAKER human heart cDNA library. The yeast was selected following the manufacturer's instructions. The pACT2-ZZaPK3′ plasmid from positive cloning was isolated and sequenced. The 3′ end ZZaPK cDNA was labeled with [α-^{32}P]dCTP and used to screen the human placenta cDNA library. The hybridization conditions for screening this library were 5× SSC and 40% formamide at 42 °C for 16 h. The final washing condition was 1× SSC at 68 °C.

Cell culture and transfection. Rat 6 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% calf serum and antibiotics (25 U/ml penicillin and 25 U/ml streptonicillin). 293T cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and antibiotics (25 U/ml penicillin and 25 U/ml streptomycin). Expression vector transfection was performed using the $CaPO_4$ method.

ZZaPK transcript analysis. mRNA from various human tissues (Clontech) was probed with full-length ZZaPK cDNA labeled using the random priming method. Hybridization was performed as described.

Immunoprecipitation and Western blot analysis. Cell lysates were prepared in IP buffer (40 mM Tris–HCl (pH 7.5), 1% NP40, 150 mM NaCl, 5 mM EGTA, 1 mM DTT, 1 mM PMSF, 20 mM NaF, proteinase inhibitors, and 1 mM sodium vanadate). Cell extracts (600 μg) were incubated with 5 μg of anti-GFP mAb (Clontech) for 6 h at 4 °C, mixed with 20 μl protein A–Sepharose suspension, and incubated for an additional hour. Immunoprecipitates were collected by centrifugation, washed three times with IP buffer plus 0.5% deoxycholate and 5× with IP buffer alone, and subjected to SDS–PAGE. Immunoblot analysis was performed with the anti-FLAG (Sigma).

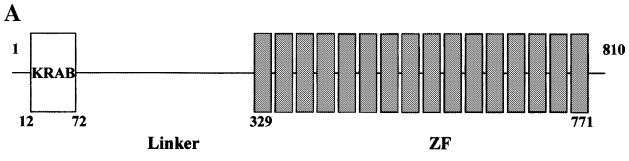
Cyc E/CDK2 activity detection. For the in vitro cyclin E-kinase assays, cyclin E was immunoprecipitated from 500 μg of total cellular protein with either polyclonal anti-cyclin E antibody (M-20; Santa Cruz Biotechnology). The immunoprecipitates were washed three times with lysis buffer and twice with kinase buffer (20 μM Tris–HCl [pH 7.4], 4 μM $MgCl_2$). The washed immunoprecipitates were then incubated with kinase buffer, 2 μmg histone H1, 1 μmM ATP, and 5 μCi [γ-^{32}P]ATP in a final volume of 16 μl for 30 min at 37 °C. The reaction products were separated on an SDS–12%–polyacrylamide gel. The gel was then dried and exposed to X-ray film.

Cell cycle analysis. Cells were grown for 24 or 72 h in the presence of doxycycline to induce the ectopic genes. Cells were then plated to 40% confluency (2.5×10^6/plate) on 100-mm plates. Cells were filtered to remove cell aggregates (Falcon filter top tubes) and analyzed for DNA content using fluorescence-activated cell sorting (FACS) analysis with a FACSCalibur flow cytometer (BD PharMingen). Data were analyzed using the Cell Quest (BD PharMingen and ModFit (Verity) analysis software.

Results

Isolation of a ZAK-interacting protein, ZZaPK, which contains a KRAB domain and 16 zinc finger motifs

A fusion protein containing the GAL4 DNA-binding domain and ZAK was used as the bait in the yeast two-hybrid screen for proteins associated with ZAK from a human heart cDNA library because ZAK is well expressed in heart tissue. Eighty-one clones that specifically interacted with the bait were identified. Restriction enzyme and nucleotide sequence analysis revealed that five of these clones contained a 3.8-kb cDNA. The cDNA contained a 247-amino acid open reading frame, followed by a stop codon. Additional cDNA clones were obtained from human placenta cDNA library screening using a ZZaPK cDNA insert as the radio-labeled probe. Seven cDNA clones were isolated and characterized. cDNA was isolated from one of these clones that encoded a novel human protein of 810 amino acids bearing 16 consecutive Krüppel-type C2H2 zinc finger motifs (Fig. 1A). It is unclear whether the cloned cDNA included the full 5′-end of this gene, a mixture (liver, placenta, thyroid, fetus, and stomach; Stratagene) of the



B

MNKVEQKSQES**VSFKDVTVGFTQEEWQHLDPSQRALYRDVMLENYSNLV**
SVGYCVHKPEVIFRLQQGEEPWKQEEEFPSQSFPVWTADHLKERSQENQSK
HLWEVVFINNEMLTKEQGDVIGIPFNVDVSSFPSRKMFCQCDSCGMSFNTVSE
LVISKINYLGKKSDEFNACGKLLLNIKHDETHTQEKNEVLKNRNTLSHHEETL
QHEKIQTLEHNFEYSICQETLLEKAVFNTQKRENAEENNCDYNEFGRTLCDSS
SLLFHQISPSRDNHYEFSDCEKFLCVKSTLSKPHGVSMKHYDCGESGNNFRRK
LCLSHLQKGDK*GEKHF*E**C**NE**C**GKAFWEKS**H**LTR**H**QRVHT*GQKPF*Q**C**NE**C**E
KAFWDKSNLTK**H**QRS**H**T*GEKPF*E**C**NE**C**GKAFSHKSALTL**H**QRT**H**T*GEKPY*Q
CNA**C**GKTFCQKSDLTK**H**QRT**H**T*GLKPY*E**C**YE**C**GKSFRVTSHLKV**H**QRT**H**T*G*
*EKPF*E**C**LE**C**GKSFSEKSNLTQ**H**QRI**H**I*GDKSY*E**C**NA**C**GKTFYHKSLLTR**H**QII**H**
T*GWKPY*E**C**YE**C**GKTFCLKSDLTV**H**QRT**H**T*GQKPF*A**C**PE**C**GKFFSHKSTLSQ**H**
YRT**H**T*GEKPY*E**C**HE**C**GKIFYNKSYLTK**H**NRT**H**T*GEKPY*E**C**NE**C**GKAFYQKSQ
LTQ**H**QRI**H**I*GEKPY*K**C**NE**C**GKAFCHKSALIV**H**QRT**H**T*QEKPY*K**C**NE**C**GKSFC
VKSGLIF**H**ERK**H**T*GEKPY*E**C**NE**C**GKFFRHKSSLTV**H**HRA**H**T*GEKSC*Q**C**NE**C**G
KIFYRKSELAQ**H**QRS**H**T*GEKPY*E**C**NT**C**RKTFSQKSNLIV**H**QRR**H**IGENLMNE
MDIRNFQPQVSLHNASEYSHCGESPDDILNVQ

Fig. 1. Amino acid sequence and the possible structure of the ZZaPK protein. (A) Schematic representation of the primary structure of ZZaPK. The numbers in the diagram represent amino acid residue numbers and delineate the boundaries of the indicated domains. (B) Conceptually translated amino acid sequence of the ZZaPK protein. Bold letters in N-terminal denote the KRAB domain (12–72). There are 16 tandemly repeated Krüppel-type zinc fingers (329–771) near the C-terminal of this protein. The Krüppel-type zinc fingers are underlined.

human 5′-RACE cDNA library was employed. It was unable to extend this cDNA to the 5′ using this technology. This cloned cDNA was predicted to encode a novel protein with Krüppel-type zinc fingers designed as ZZaPK (zinc finger and ZAK associated protein with KRAB domain) (Figs. 1A and B). The open reading frame of this cDNA encodes a putative polypeptide of 810 amino acids, with a calculated molecular mass of 94.3 kDa (GenBank Accession No. AY184389). To ascertain the expression and the molecular weight of ZZaPK, 293 cells were transfected with either GFP or GFP-tagged ZAK plasmids. The expressed proteins were determined through Western blot analysis using an antibody specific for GFP. A band at a molecular weight of 121 kDa was detected. The molecular weight of the GFP protein is about 27 kDa, therefore the molecular weight of ZZaPK is about 94 kDa (Fig. 2B). The presence of these functional motifs (zinc fingers and KRAB domain) within the predicted coding sequence suggests that ZZaPK encodes a transcription factor. Analysis of the predicted amino acid sequence indicates that ZZaPK contains KRAB A and B motifs at its amino termini. At the carboxyl terminus of the predicted ZZaPK protein, 16 tandemly repeated Krüppel-type zinc fingers were found. A linker motif of 256 amino acids separates the KRAB domain and the zinc fingers. Based on the DNA and amino acid sequence, ZZaPK seems to be a novel member of the KRAB family of transcriptional factors.

ZAK mRNA tissue distribution

To ascertain the expression pattern of the ZZaPK gene, the endogenous expression of ZAK mRNA was studied in various human tissues. Northern blot analysis of various human tissues demonstrated that the major transcript for ZZaPK is 5.5 kb (Fig. 2A). ZZaPK gene expression could be detected in all tissues examined. The wide ZZaPK expression suggests that it might not have cell type-specific functions. Moreover, ZAK is expressed highly in the heart and that both genes work together might contribute to specific functions in this tissue.

Interaction between ZAK and ZZaPK in mammalian cells

To establish that the interaction identified by the yeast two-hybrid system can occur in mammalian cells, GFP-tagged ZAK was coexpressed in 293 cells with FLAG-tagged ZZaPK. Protein complexes were immunoprecipitated using the anti-GFP antibody and coprecipitated FLAG-ZZaPK was probed using M2 monoclonal antibody. As shown in Fig. 3A, FLAG-ZZaPK could be detected only when the cells expressed both FLAG-ZZaPK and GFP-ZAK plasmids. This result demonstrates that ZAK proteins might interact with ZZaPK in mammalian cells. Moreover, the double negative controls when cells expressed both pFLAG and pGFP demonstrated that FLAG and GFP do not interact directly.

To map the region on ZAK required for binding to ZZaPK, serial ZAK C-terminal deleted mutants were generated, carrying an EGFP epitope coexpressed with FLAG-tagged ZZaPK in 293 cells. FLAG-ZZaPK was pull-down only when the cells expressed ZAK mutants that have the SAM domain (Fig. 3B). Therefore, the SAM domain was required for binding to ZZaPK in this assay. The leucine-zipper alone was not sufficient to bind

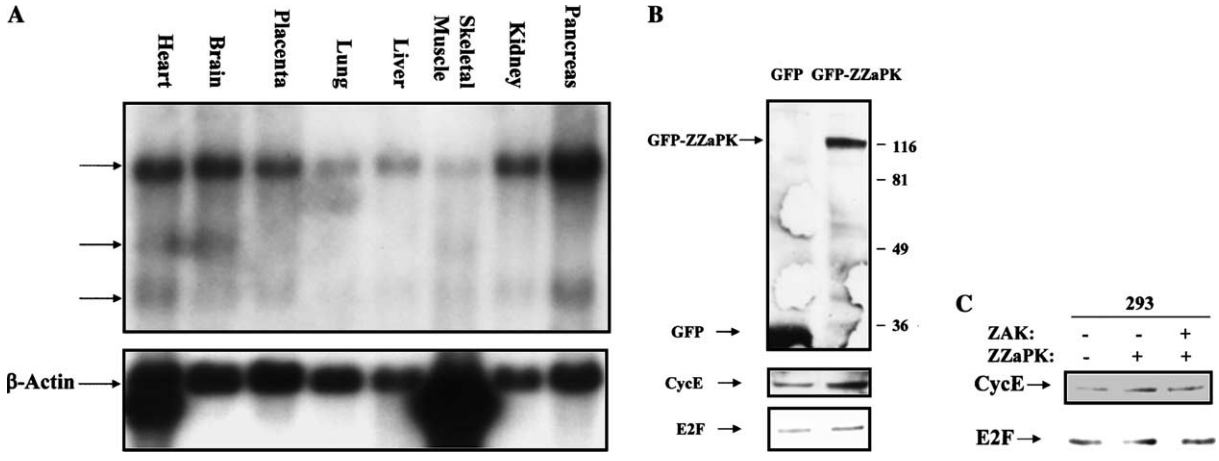


Fig. 2. ZZaPK is widely expressed in human tissues and expression of epitope-tagged ZZaPK in 293 cells. (A) ZZaPK mRNA expression as shown by Northern blot analysis from various human tissues using ZZaPK cDNA as a probe. A human multiple tissue Northern blot containing poly(A)$^+$ RNA was probed with a full-length ZZaPK fragment and a human β-actin cDNA. (B) The GFP-tagged ZZaPK or GFP plasmid (20 μg) was transiently transfected into 293 cells through a calcium phosphate transfection protocol. Transfectants were lysed 48 h after transfection and the lysates were resolved using SDS–PAGE, followed by immunoblot analysis with a GFP, cyclin E, and E2F1 antibodies. (C) The GFP-tagged ZZaPK and ZAK plasmids were transiently cotransfected into 293 cells for 48 h and the expression of cyclin E and E2F was determined through immunoblotting.

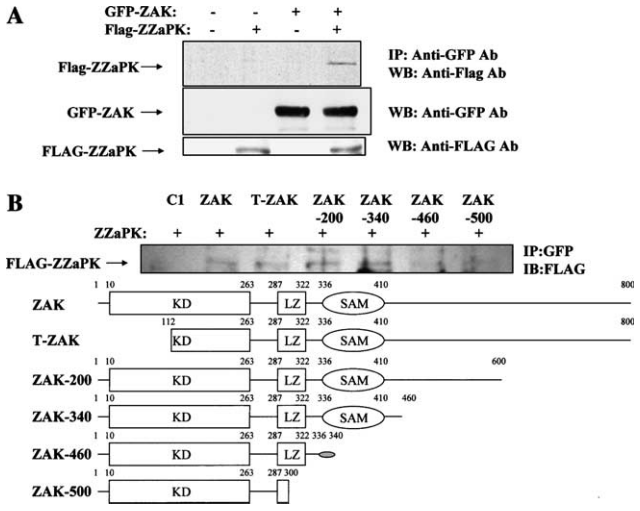


Fig. 3. Specific interaction between ZAK and ZZaPK. (A) GFP- and FLAG epitope-tagged forms of ZZaPK were transiently expressed in 293 cells either alone or together as indicated. The anti-GFP antibody was used for immunoprecipitation and the anti-FLAG antibody for the Western blot. The middle and bottom panels are Western blots, demonstrating that either GFP- or FLAG-ZZaPK was expressed in both the single and double transfections. (B) Specific binding of full-length ZZaPK to different ZAK mutants. Schematic diagrams indicate the full-length and different deleted mutants of ZAK.

ZZaPK. Taken together, these results indicate that the kinase-domain, leucine-zipper nor the less characterized C-terminal region in ZAK is required for ZZaPK binding. The SAM domain, however, might be required.

ZZaPK can suppress ZAK-dependent cell growth arrest

A previous study demonstrated that ZAK expressing cells showed a decreased growth rate in comparison with parental cells. Because ZAK and ZZaPK interact in vivo, it was of interest to examine the growth effect of these two proteins when coexpressed in cells. Tetracycline-responsible transactivator regulated ZAK-expressed or control Rat6 cells were transfected with pGFP-ZZaPK. A selective pBabepuro plasmid and stable ZZaPK-expressed cells were generated under puromycin selection. A set of 5000 cells that expressed ZAK, ZZaPK, or ZAK and ZZaPK proteins were cultured in six-well dishes. The cell growth rate was determined every 24 h. The ZAK expressing cells showed decreased growth rate in comparison with parental cells whereas the ZZaPK-expressing cells showed no growth rate effect. However, cells expressing both of ZAK and ZZaPK proteins showed growth rate comparable to parental cells (Fig. 4A). This result indicated that ZZaPK protein expression in cells might be a growth promoted counteraction to the negative effect of ZAK.

As indicated in a previous study, ZAK activation might be involved in the regulation of G_2 checkpoint control. It is of interest to determine whether ZZaPK is able to overcome G_2/M stage cell cycle arrest. ZAK-expressed cells had a significantly higher percentage of cells in the G_2/M phase of the cell cycle stage (39.04%) while cells that expressed both ZAK and ZZaPK proteins had a lower G_2/M phase (10.78%). Moreover, ZAK and ZZaPK-expressed cells showed increased G_1 phase (67.86%) in the cell cycle compared with the ZAK-expressed cells (46.50%). The cell cycle stage in ZAK and ZZaPK expressing cells is similar to parental cells in which there was 8.71% in G_2/M phase and 79.82% in G_1 phase (Fig. 4B). This observation suggested that ZZaPK might regulate the cell cycle entrance. However, ZZaPK expression cells had a significantly increased S phase (28.44%) compared to parental cells (11.47%) and increased G_2/M (28.54%) compared to the parental cells (8.71%) (Fig. 4B). These results showed that ZZaPK expression might stimulate entrance into the S phase.

ZZaPK stimulates cyclin E expression

The low proliferative capacity of ZAK-expressed cells was due to the decreased cyclin E level. Cyclin E expression was therefore tested in ZZaPK expressing cells. The ZZaPK-expressing cells were able to increase the cyclin E expression level in both ZAK and control background cells (Fig. 4C). In contrast, there was no significant change in cyclin A expression level in these cells. This result indicated that ZZaPK expression might specifically lead to increasing the cyclin E expression. ZZaPK is able to induce cyclin E expression in stable cell lines and transient transfected 293 cells (Fig. 2B).

The cyclin E expression event was detected in ZZaPK expressing cells. Cyclin E-dependent kinase activity was analyzed using immunoprecipitating cyclin E from these cells and then performing in vitro kinase assays with histone H1 as the substrate. The cyclin E/Cdk2 activity was low in ZAK-expressing cells. Alternatively, both ZZaPK-expressing cells produced higher cyclin E/Cdk2 activity (Fig. 4C). Taken together, these data suggest that ZZaPK restores the cell cycle entrance in ZAK-expressing cells through the increasing cyclin E expression.

Regulation of pRB phosphorylation and E2F expression by ZZaPK

The induction of cyclin E might or might not be directly regulated by the ZZaPK protein. The E2F transcription factor plays a key role in the timely activation of cyclin E expression during mammalian cell cycle progression. It is therefore important to detect the E2F expression level in these cells. E2F expression was decreased in ZAK-expressing cells in comparison to the control cells. The ZZaPK-expressing cells were able to increase E2F expression in both

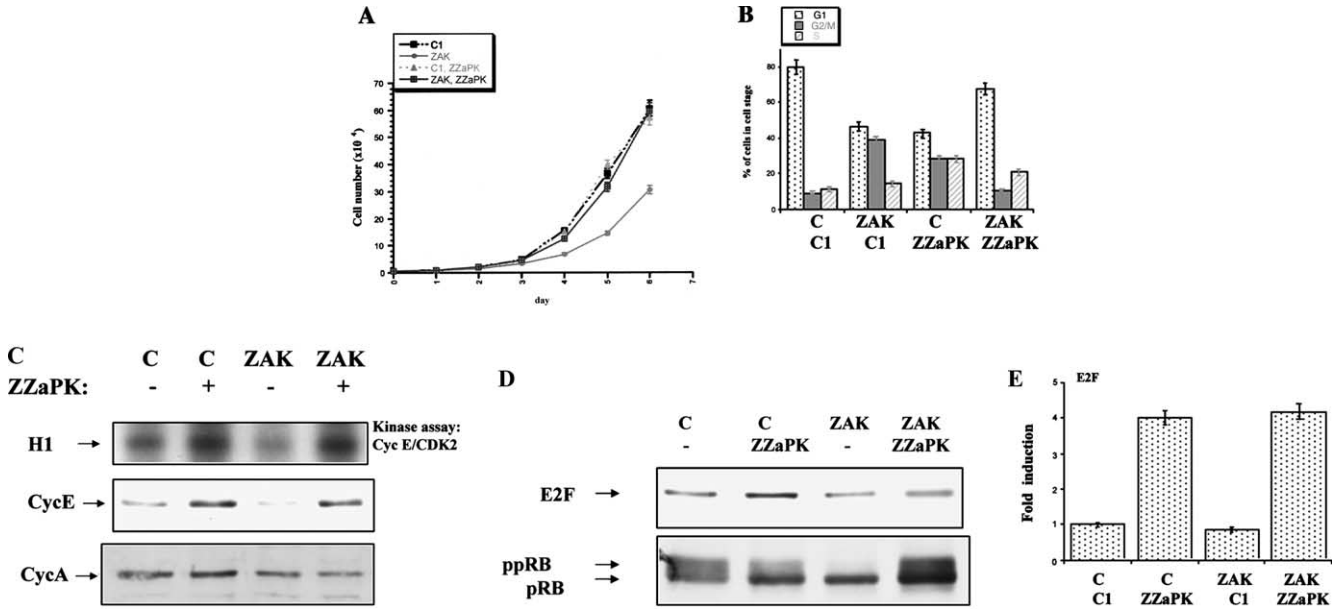


Fig. 4. Bilogical and biochemical studies on ZZaPK restore the cell cycle re-entrance of ZAK-expressing cells. (A) Growth curves of Rat6 or Rat6-ZAK cells with or without expression of ZZaPK. Stable Rat6 or Rat6-ZAK cell lines, which stable expressed wild-type ZZaPK or control vector, were seeded at a original density of 5000 cells per six-well dish in the presence of 1 μg/ml doxycycline and cell number was counted at the indicated time. (B) These stable Rat6 cell lines express the indicated genes for 24 h and then analyzed by FACE. The percentage of cells in the cell cycle phases was calculated by the ModFit software. Experiments were performed three times and gave similar results. (C) Steady-state levels of cyclin E or cyclin A proteins and cyclin E/CDK2 activity in these stable Rat6 cell lines. (D) Steady-state levels of E2F and RB proteins in these stable Rat6 cell lines. (E) ZZaPK mediated the induction of E2F activity in these Rat6 cells. Cells (2.5×10^5 were seeded in six-well plates and transfected with 1 μg of the pE2F-luciferase. Cell lysates were collected 48 h after transfection and measured for luciferase activity. Experiments were performed four times. Representative data are shown.

the control and ZAK background cells (Fig. 4D). ZZaPK is able to induce E2F expression in stable cell lines and transient transfected 293 cells (Fig. 2B). The above data suggested that ZAK and ZZaPK might produce counteractive signals to E2F and cyclin E expression. To address this question, 293 cells were transient transfected with ZZaPK or ZAK and ZZaPK and the E2F and cyclin E expression was determined using Western blots. E2F and cyclin E expression was induced using ZZaPK where ZAK is able to suppress this induction (Fig. 2C). The tumor suppressor gene, pRB, controls cell growth through the ability to block the action of E2F. Western blots of extracts derived from these cells were probed with an antibody to pRB. The hypophosphorylated form was present in ZAK-expressing cells. Conversely, both ZZaPK-expressing cells exhibited hyperphosphorylated pRB (Fig. 4D). These data suggest that both ZZaPK-expressing cells had a higher expression level of E2F, with the E2F proteins mainly in the active form. To address this question, it is reasonable to determine whether ZZaPK-expressing cells would induce an E2F-dependent promoter. These stable cell lines were transfected with E2F-luciferase plasmid in the presence of tetracycline. The E2F-dependent luciferase activity was increased in both ZZaPK-expressing cells (Fig. 4E). Taken together these data indicated that the expression of E2F was induced by ZZaPK where ZAK counteracts this induction.

Discussion

Using the yeast two-hybrid system, a novel ZAK-associated Krüppel-type C2H2 zinc finger motif cDNA was identified and designed as ZZaPK. This protein is widely expressed in many tissues and interacts with ZAK in yeast and in vivo. The ZAK C-terminal deletion experiment shows that the SAM domain within ZAK is required for ZZaPK binding. Moreover, ZZaPK is able to restore the cell cycle entrance for ZAK-expressing cells through increasing the expression levels of E2F and cyclin E.

A question concerning the role of ZZaPK in the induction of E2F expression is emerging. The presence of zinc fingers and the KRAB domain within the ZZaPK coding sequence suggests that ZZaPK encodes a transcription factor. It is still unclear if ZZaPK might directly bind to its recognition sequence and mediate the transcription of E2F. A further study is underway to determine the consensus ZZaPK DNA-binding sequence, which might be useful in identifying the target

genes regulated by ZZaPK. The KRAB gene family has been suggested as transcriptional repressors because the KRAB domain itself harbors the transcriptional repressor activity for these proteins [20–23]. It is, therefore, possible that ZZaPK does not directly mediate the expression of E2F. Whether a direct or indirect mechanism is involved in ZZaPK-mediated induction of E2F expression will be important questions to address experimentally.

The finding that ZZaPK is widely expressed suggests that its potential role in cell cycle regulation might be independent of the cell type. ZZaPK counteracts ZAK-mediated cell cycle arrest and that both of these proteins are associated in vivo has raised a question of whether ZZaPK activity might be negatively regulated by ZAK. The transient transfection experiment might partially answer this question. 293 cells transfected with plasmid harbored ZZaPK increased the expression level of E2F and cyclin E where ZAK is able to suppress this induction in the cotransfection experiment. Because ZAK belongs to MAP3K, it is of interest to determine if the function of ZZaPK would be regulated using post-translated modification, especially using ZAK kinase activity. It is important to study the liner relationship between these two proteins.

In this study the RB protein is hypophosphorylated in ZAK-expressing cells. Conversely, ZZaPK increases its phosphorylation. This work demonstrated that the function of RB was inhibited and its ability to interact with E2F was abolished. Moreover, ZZaPK is able to increase E2F expression. The role of E2F transcription factor activity in controlling the transition from G_1 to the S phase reveals that ZZaPK-expressing cells are able to stimulate cells entering the S phase (Fig. 4B). E2F also directs the synthesis of cyclin E, creating the kinase activity (Fig. 4C). It will be important to confirm the mechanism by which ZZaPK functions to regulate E2F expression.

It was proven that the SAM domain within ZAK provides for the ZAK–ZZaPK interaction. However, the binding region in ZZaPK must be addressed for this kind of interaction. The first ZZaPK cDNA clone from the yeast two-hybrid experiment indicates that the C-terminal 247-amino acid might be involved in this interaction. This region contains the consecutive Krüppel-type C2H2 zinc finger motifs. It is, therefore, suggested that zinc finger motifs might be involved in the interaction with the SAM domain.

In conclusion, a novel Krüppel-type zinc finger protein, ZZaPK, was cloned to interact with ZAK. ZZaPK, when it is expressed in cells, is growth promoted and might lead to increased E2F expression and induce cyclin E/CDK2 activity. The ZZaPK counteracts ZAK-mediated cell cycle arrest through the regulation of E2F expression and cyclin E/CDK2 activity.

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