Zinc finger protein HZF1 promotes K562 cell proliferation by interacting with and inhibiting INCA1

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Abstract. Previously, we characterized a zinc finger protein gene HZF1 (ZNF16) and demonstrated that it played a significant role in the erythroid and megakaryocytic differentiation of K562 cells by knockdown of the gene. In this study, we examined the effect of HZF1 on the proliferation and apoptosis of K562 cells and identified the possible mechanism for this effect. By lentivirus-mediated gene transfer, we obtained stable K562 transductants with HZF1 overexpression (K562/WPXL-HZF1) and stable control transductants (K562/WPXL). Significantly rapid cell amplification was observed in K562/WPXL-HZF1 cells compared to K562/WPXL cells. The cell cycles of the two transductants were analyzed and the results demonstrated that HZF1 overexpression promoted the S to G2/M phase transition. Additionally, we found that the overexpression of HZF1 slightly inhibits the apoptosis of K562 cells induced by sodium arsenate. Furthermore, using a yeast two-hybrid (Y2H) system we identified the HZF1-interacting proteins and screened 29 potential binding partners of HZF1. Using a co-immunoprecipitation (Co-IP) assay, we confirmed the interaction between HZF1 and the inhibitor of cyclin-dependent kinase (CDK) interacting with cyclin A1 (INCA1), and proved that this interaction leads to the inhibition of INCA1 function, which rescued the activity of CDK2 inhibited by INCA1. In conclusion, our results identified novel functions of the HZF1 gene and revealed a possible mechanism through which HZF1 affects K562 cell proliferation.

Introduction

The C_2H_2-type zinc finger proteins that consist of tandem arrays of two or more zinc finger motifs are one of the most abundant proteins in eukaryotic cells and are involved in all facets of cellular activities including cell growth, proliferation, apoptosis and differentiation (1-6). Previously, we identified and characterized a C_2H_2 type zinc finger protein gene HZF1 (ZNF16) by screening a human bone marrow cDNA library (7). The full reading frame of the HZF1 gene encodes a 670 amino-acid peptide, and the C-terminal of this peptide contains 17 uninterrupted zinc fingers including 15 typical and 2 atypical C_2H_2 zinc finger motifs, and TGEKPYE repeats between any two zinc fingers (7). By using an antisense method and a RNA inference assay, we previously showed that repression of the intrinsic expression of HZF1 reduced hemin-induced erythroid differentiation and phorbol myristate acetate (PMA)-induced megakaryocytic differentiation of K562 cells, suggesting its significant role in erythroid and megakaryocytic differentiation (7). The Northern blot assay revealed that HZF1 was extensively expressed in human tissues and cell lines (7), indicating that HZF1 may be involved in the regulation of certain significant cellular functions. In this study, HZF1 overexpression was found to accelerate the proliferation of K562 cells by facilitating the cell cycle phase transition from the S to G2/M phase, and slightly inhibited the apoptosis of K562 cells.

C_2H_2 type zinc finger domains were originally identified as DNA-binding domains, and were typically assumed to be involved in DNA binding. Additionally, HZF1 was found to be located in the cell nucleus and may be a transcriptional activator (8). However, a growing body of evidence suggests a crucial and widespread role for this domain in protein binding. Examples of zinc fingers support DNA and protein interactions, and may be found in well-known DNA-binding proteins such as Sp1 and GATA1 (9). C_2H_2 zinc finger protein-protein interactions are more abundant than previously appreciated, more plastic than their DNA-binding counterparts, and more variable and complex in their interactions surfaces (10). Using a yeast two-hybrid (Y2H) system we identified the HZF1-interacting proteins and screened 29 potential binding partners of HZF1. One of these partners is the inhibitor of cyclin-dependent kinase (CDK) interacting with cyclin A1 gene (INCA1), the interaction partner and substrate of the cyclin A1-CDK2 or cyclin A2-CDK2 complex that played a negative role in cell cycle process regulation by inhibiting CDK interacting with cyclin A1 (11). We therefore examined and confirmed the interaction between INCA1 and HZF1 by co-immunoprecipitation (Co-IP) and proved that the interaction facilitates the cell cycle process of K562 cells.

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Material and methods

Cell line and culture. The chronic myelogenous leukemia cell line K562 was purchased from the Cell Center of the Institute of Basic Medical Science, Chinese Academy of Medical Sciences.The 293T/17 cell line was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were grown in Dulbecco's modified Eagle's medium (DMEM, Gibco-BRL, Grand Island, NY, USA) containing 10% (v/v) fetal bovine serum (FBS, Gibco-BRL), penicillin (100 U/ml), streptomycin (100 μg/ml) and 2 mM L-glutamine at 37˚C and 5% CO_2.

Construction of lentiviral vectors and production of lentivirus. The self-inactivating transfer vector plasmid pWPXL (12), which expresses enhanced green fluorescent protein (EGFP) under the control of EF1α promoter, was obtained from Addgene (South San Francisco, CA, USA). The coding sequence of HZF1 was amplified from pcDNA3.1-HZF1 (7) by PCR with the primers 5'-ACAGGATCCATGGAGCTCTCAGTTCCAGGAC-3' and 5'-AGACACGCGTTCCCTGGTGTGAATCAACT-3'. The amplified fragment was sequenced and digested with *Bam*HI and *Mlu*I followed by cloning into the pWPXL at the *Bam*HI and *Mlu*I sites joining the 5'-end of EGFP to obtain the recombinant plasmid pWPXL- HZF1.

The lentiviral supernatants were prepared by calcium phosphate-mediated DNA cotransfection of 293T/17 cells as previously described (12). Briefly, 293T/17 cells were plated on poly-L-lysine-coated 10-cm plates at $3x10^6$ cells/plate in DMEM with 10% FBS and allowed to grow for 24 h prior to transfection with 10 μg of the transfer vector plasmid pWPXL-HZF1 or pWPXL, 10 μg of pSPAX2 packaging plasmid and 2 μg of pMD.2G (VSV-G) envelope plasmid (12). Eight hours following the application of the DNA precipitate, the cells were rinsed three times with phosphate-buffered saline (PBS), and then cultured in fresh DMEM containing 10% FBS. The lentiviral supernatants were collected 48 h following the application of fresh medium and then filtered through a 0.2 μm syringe filter and stored at -80˚C.

To determine the titers of the lentiviral supernatants, 293T/17 cells were plated at $1x10^5$ cells/well in 12-well plates and allowed to adhere for 4 h. The medium was then removed and replaced with 1 ml of supernatants diluted at 1:100, 1:1000 and 1:10,000. The viral supernatants remained on the cells overnight and were then replaced with fresh medium. The titers were determined at 72 h following the addition of vector by analyzing the expression rate of EGFP using a fluorescence microscope (Nikon, Japan). The viral titers (IU/ml) were calculated according to the following equation: EGFP positive rate x dilution factor x 10^5. Titer assays were performed in duplicate.

Generation of the stable K562 cell transductants by lentivirus infection. K562 cells were plated at $1x10^4$ cells/well in 24-well plates and infected with 10 MOI of the lentiviral supernatants, respectively. The medium was removed and replaced with 1 ml fresh medium following culture for 24 h. Following proliferation for a number of passages, the infected cells that stably expressed EGFP were purified by sorting flow cytometry and we obtained a stable transductant with HZF1 overexpression (K562/WPXL-HZF1) and a stable control transductant (K562/WPXL).

RNA isolation and quantitative RT-PCR. Total RNA was extracted from the cell harvest using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The RNA was quantified by absorbance at 260 nm. The cDNA was synthesized by M-MLV reverse transcriptase (Invitrogen) from 2 μg of total RNA with Oligo(dT)18 as the primer. Quantitative RT-PCR was carried out in an ABI PRISM 7500 real-time PCR System (Applied Biosystems, Foster City, CA, USA) using a SYBR Premix Ex Taq kit (Takara, Dalian, China) according to the manufacturer's instructions. The comparative Ct method was used to quantify target genes relative to the endogenous control (GAPDH). For each individual analysis, one of the samples was designated as the calibrator, and allocated a relative value of 1.0. Quantities were expressed as n-fold relative to the calibrator. Real-time primer sequences were: GAPDH amplification (forward, 5'-ATGGGGAAGGTGAAGGTCG-3'; reverse, 5'-GGGGTCATTGATGGCAACAATA-3'); HZF1 amplification (forward, 5'-CCCGTGTGAGAGATGCTCC-3'; reverse, 5'-CTGGCTGCTGATAGTGAGGG -3').

Cell apoptosis assay. The PE annexin V apoptosis detection kit I (BD Pharmingen, Rockville, MD, USA) was used to detect the effect of HZF1 on the apoptosis of K562 cells. Briefly, $1x10^6$ K562/WPXL and K562/WPXL-HZF1 cells were exposed to various concentrations of sodium arsenate for 12 h, respectively. The cells were then collected and washed twice with cold PBS prior to re-suspension of cells in 1X binding buffer at a concentration of $1x10^6$ cells/ml. A total amount of 100 μl of the solution ($1x10^5$ cells) was transferred to a 5 ml culture tube. Then 5 μl of PE annexin V and 7-AAD were added, the cells were gently vortexed and incubated for 15 min in RT (25˚C) in the dark, while 400 μl of 1X binding buffer was added to each tube and the samples were analyzed by flow cytometry within 1 h.

Cell cycle assay. The cell cycle was partitioned by measuring intracellular DNA via flow cytometry. Briefly, cells were synchronized by serum starvation for 8 h, then cultured in completed medium and harvested at various time points. The harvested cells were washed twice with PBS and then fixed in 75% ethanol at 4˚C overnight. Cells were washed and incubated in RNaseA (20 μg/ml) at 37˚C for 30 min, and stained with propidium iodide (PI, 0.5 mg/ml) at 4˚C for 30 min. Cells were washed and resuspended in 500 μl PBS followed by detection of the DNA content using a Becton-Dickinson flow cytometer.

Cell proliferation assay. For the cell proliferation assay, $1x10^5$ cells were plated in a 3.5 cm dish and cultured in complete medium at 37˚C and 5% CO_2. Cells were counted using a cell counting chamber at interval time and the cell growth curve was drawn according to the cell numbers at various time points.

Identification of HZF1-interacting proteins by the Y2H system. To identify HZF1-interacting proteins, a Y2H screen

was performed using the Matchmaker™ Gal4 two-hybrid system 3 (Clontech). Experiments were carried out according to the manufacturer's instructions (Clontech, Yeast Protocols Handbook, Matchmaker™ two-hybrid system 3). In brief, a human bone marrow cDNA library was cloned into the pACT2-AD vector (Clontech). The zinc fingers region of HZF1 was cloned into a pBridge-BD vector and served as bait for the library translation products. The plasmids were co-transformed into the yeast strain AH109 (Clontech). The transformed yeast cells were grown on high stringency selection plates [-Met, -Leu, -Trp, -His, -Ade, +5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (+X-gal)]. The cDNA inserts in the positive yeast colonies were amplified by nested PCR. Sequences were analyzed by alignment to the NCBI databases.

Co-immunoprecipitation and Western blot analysis. For the Western blot analysis, total proteins were extracted from cells using ice-cold lysis buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 1 mM sodium orthovanadate, 1 mM sodium fluoride and 1 mM EDTA), respectively. Total proteins (20 μg) were segregated on a 10% SDS-PAGE gel and then transferred to polyvinylidene difluoride membranes (Amersham, UK). The membrane was incubated with specific primary antibodies after being blocked with 5% degrease milk powder, washed and incubated with peroxidase-linked secondary antibodies, while the membranes were exposed to ECL hyper film (Amersham).

For co-immunoprecipitation (Co-IP), 293T/17 cells were transfected with the pflag-HZF1 and/or pmyc-INCA1. Following a 36-h transfection, the cells were harvested and lysed, and the expression of target fused proteins was identified by Western blot analysis. Then, 500 μl of lysates of the transfected cells were agitated with 2 μg of flag antibody at 4˚C for 1 h, 60 μl of protein A/G-agarose beads (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were added, and the reaction mixture was agitated at 4˚C again for 2 h. Following washing with ice-cold cell lysis buffer, the bound proteins were subjected to SDS-PAGE and blotted for myc-INCA1.

Primary antibody against HZF1 was raised in rabbit as previously described (7). Additional primary antibodies used for immunoprecipitation or Western blot analysis detection were rabbit polyclonal-pRb (Abcam, UK), mouse monoclonal-Rb (Abcam), mouse monoclonal-c-Myc (Santa Cruz), mouse monoclonal-Flag (Santa Cruz) and mouse monoclonal-GAPDH (Clontech).

Results

Generation of the stable K562 cell transductants. By lentiviral vector-mediated gene transfer we obtained stable K562 transductants with HZF1 overexpression (K562/WPXL-HZF1) and stable control K562 transductants (K562/WPXL). The relative expression of HZF1 was identified by quantitative real-time PCR, and the results demonstrated that the expression of HZF1 in K562/WPXL-HZF1 was 47.8 times greater than that in K562/WPXL. Western blot analysis revealed a significant increase of the HZF1 protein level in K562/WPXL-HZF1 cells compared to K562/WPXL. These results confirmed the overexpression of HZF1 in K562/WPXL-HZF1 (Fig. 1).

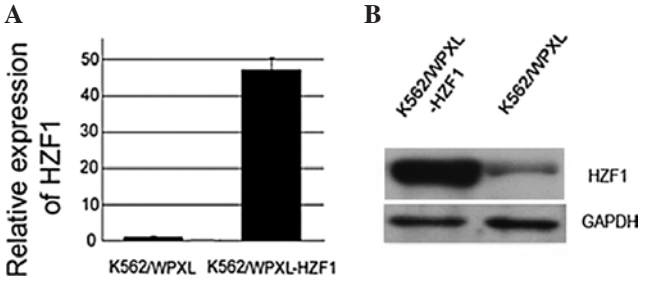

Figure 1. Identification of HZF1 overexpression in stable transformant K562/WPXL-HZF1. (A) Quantitative PCR assay. Comparative real-time PCR was performed in triplicate to detect the expression of HZF1 in K562 cells. The expression level of HZF1 was normalized by GAPDH and expressed as relative folds (the mean ± SD). (B) Western blot assay with anti-HZF1 antibody.

HZF1 overexpression slightly inhibits apoptosis of K562 cells. To detect the effect of HZF1 on the apoptosis of K562 cells, HZF1 overexpression in K562 and control cells was treated with 0, 2.5 and 5 nM sodium arsenate for 12 h, respectively. Annexin V staining-based apoptosis detection was then performed. The results showed that the percentage of cells undergoing apoptosis were elevated with the increasing dose of sodium arsenate treatment in the two cells. The results also suggest that the overexpression of HZF1 slightly inhibits the apoptosis of K562 cells, judging by the fact that the apoptosis of K562/WPXL-HZF1 cells was always lower (by approximately 5%) than that in control cells when compared with the same concentration of sodium arsenate-treated K562/WPXL-HZF1 and K562/WPXL cells (Fig. 2).

HZF1 overexpression promotes proliferation of K562 cells by facilitating the phase transition from the S to G2/M phase of the cell cycle. We identified the role of HZF1 in K562 cell proliferation with a cell growth curve, and observed an accelerated proliferation of the K562/WPXL-HZF1 cells compared with the K562/WPXL cells (Fig. 3). We then determined the effects of HZF1 on the K562 cell cycle. By comparing the proportion of cells in the G and S phases between K562/WPXL-HZF1 cells over-expressing HZF1 and the control K562/WPXL cells, we found that the proportion of cells in the G2/M phase in K562/WPXL-HZF1 was higher than that in K562/WPXL cells, suggesting that HZF1 overexpression promotes the S to G2/M transition in the K562 cell cycle (Fig. 4). Taken together, these results demonstrated that HZF1 overexpression promotes the proliferation of K562 cells by facilitating the S to G2/M phase transition of the cell cycle.

Identification of HZF1 interaction partners. The mechanism by which HZF1 functions remains unclear due to a lack of information about interactions at the molecular level. To identify HZF1 interaction partners, we employed the Y2H to screen a human bone marrow cDNA library. To minimize the probability of false positive clones, we only expressed the zinc finger region of HZF1 as bait since the transcriptional activation effect of the non-zinc finger region of HZF1 was observed (8). A total of 29 sequences for putative HZF1 interaction partners were identified. Among them, 23 proteins are known functional genes, which are associated with the regulation of cell cycle, differentiation, transcription

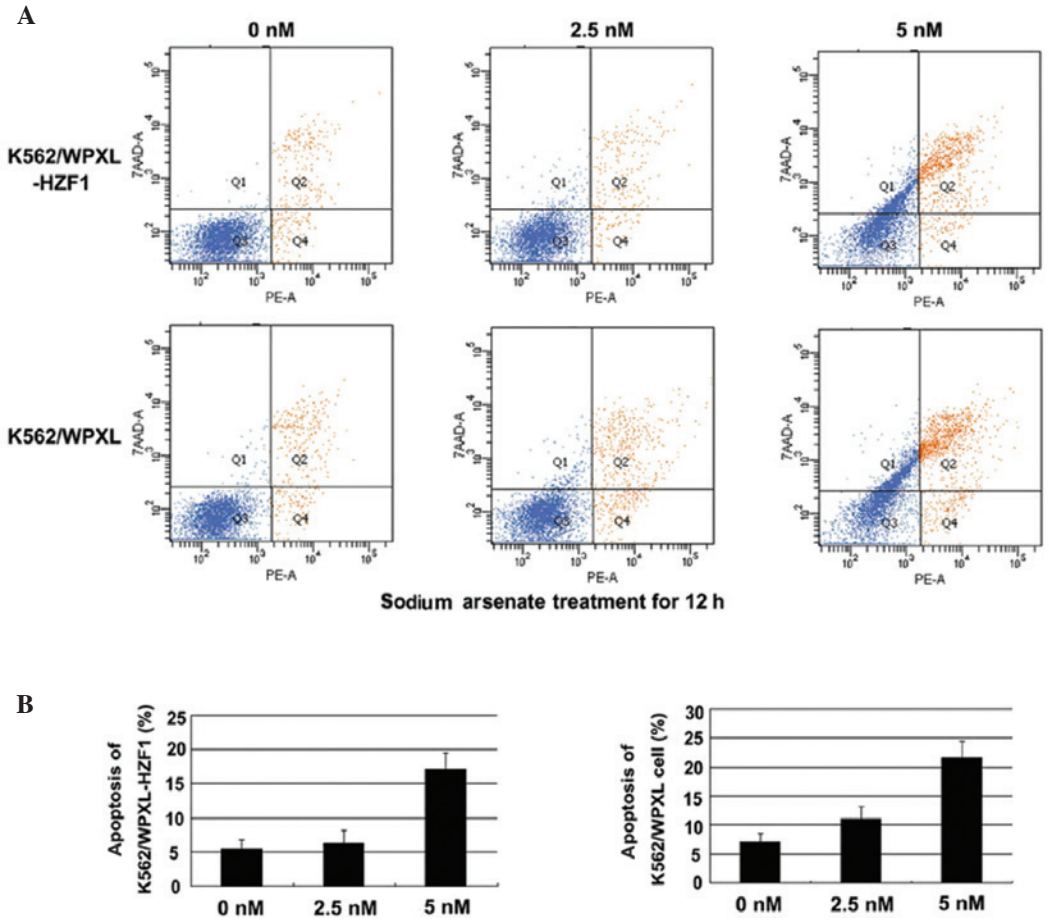

Figure 2. HZF1 overexpression slightly inhibits the apoptosis of K562 cells. (A) HZF1 overexpression in K562 and control cells was treated with 0, 2.5 and 5 nM As_2O_3 for 12 h, respectively. Annexin V staining-based apoptosis detection was then performed by flow cytometry. (B) Statistical apoptosis assay results of K562/WPXL and K562/WPXL-HZF1 cells.

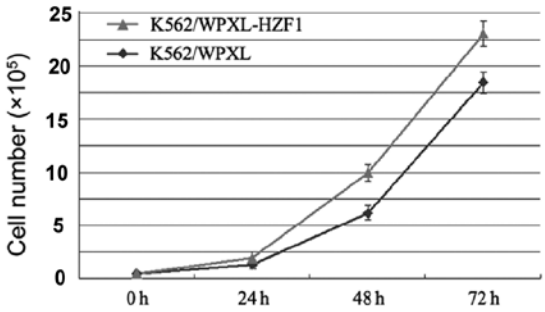

Figure 3. Growth curves of K562/WPXL and K562/WPXL-HZF1 cells. Identical numbers of cells from the two stable K562 transductant pools were incubated in fresh DMEM medium and cultured. Cells were counted at the indicated times. Cell numbers (the mean ± SD) from two independent experiments, each measured in duplicate, are shown. A marked more rapid cell proliferation was observed in K562/PWPXL-HZF1 compared to K562/WPXL, suggesting that HZF1 overexpression promotes cell proliferation.

and translation. Additionally, 4 functionally unknown and 2 hypothetical proteins were identified as potential partners of HZF1 (Table I).

HZF1 interacts physically with INCA1 and inhibits its function. One of the screened proteins is INCA1, which is associated with regulation of the cell cycle. By Co-IP assay we further certified that INCA1 was the interaction partner of HZF1 (Fig. 5A). INCA1 is an inhibitor of CDK2 interacting with cyclin A1. To determine the effect of the interaction of HZF1 and INCA1 on CDK2 activity, we identified the phosphorylated retinoblastoma (pRb) level which may affect CDK activation, and demonstrated that the pRb level was markedly higher in HZF1 overexpressed K562 cells compared to that in the control K562 cells (Fig. 5B). These results suggest that HZF1 not only interacts with INCA1, but also inhibits the function of INCA1 and, thus, facilitates the activation of CDK2.

Discussion

The typical mammalian cell cycle consists of 4 distinct phases occurring in a well-defined order. Progression of the cell cycle transitions is controlled by the activation or inhibition of a family of serine-threonine protein kinases, the CDKs, due to their post-translational modifications (phosphorylations/dephosphorylations). The proteins known as cyclins are regularly changed with the phase transition of the cell cycle, and bind to and activate CDKs, leading to the phase transition and promoting cell proliferation. Certain proteins are known to interact specifically with, and inhibit, CDKs. These proteins are known as CDK inhibitors and lead to cell cycle arrest.

Two A-type cyclins are known to be involved in cell cycle regulation: cyclin A1 and A2. Cyclin A2, also known as cyclin

Table I. Interacting proteins with HZF1 identified by yeast two-hybrid (Y2H).

No.	Interacting partners of HZF1	Functions	
1	ACTA1	Major constituent of the contractile apparatus	Muscle differentiation
2	FHL1	Playing significant role in muscle growth and carcinogenesis	Muscle differentiation
3	EPB42	Probably has a role in erythrocyte shape and mechanical property regulation	Blood cells differentiation
4	DEFA1	Plays a role in phagocyte-mediated host defense	Blood cells differentiation
5	ZXDC	Significant regulator of MHC class I and II transcription	Blood cells differentiation
6	INCA1	Involved in cell cycle regulation	Cell cycle regulation
7	S100A8	Involved in the regulation of cell cycle progression and differentiation	Cell cycle regulation
8	Rho subfamily	Encodes a member of the Rho family of GTPases	Cell signaling transduction
9	PFN1	Regulating actin polymerization in response to extracellular signals	Cell signaling transduction
10	CEACAM8	Associated with cell adhesion	Cell adhesion
11	COL1A1	Component of type I collagen	Cell adhesion
12	COL1A2	Component of type I collagen	Cell adhesion
13	ACSL6	Catalyzes the formation of acyl-CoA from fatty acids, ATP, and CoA	Metabolism
14	FABP4	Involved in the fatty acid uptake transport, and metabolism	Metabolism
15	GMPS	Encodes an enzyme that catalyzes the amination of XMP to GMP	Metabolism
16	ATP5I	Associates with mitochondrial ATP synthesis	Metabolism
17	MT2A	Plays a role in the detoxification of heavy metals and reactive oxygen species	Metabolism
18	DYNC1H1	Encodes a member of the cytoplasmic dynein heavy chain, while dyneins are a group of microtubule-activated ATPases functioning as molecular motors	Cytoskeleton
19	SVIL	Plays a role as a high-affinity link between the actin cytoskeleton and the membrane	Cytoskeleton
20	EEF1A1	Associated with the enzymatic delivery of aminoacyl tRNAs to the ribosome	Protein synthesis
21	RPL12	Associated with protein synthesis	Protein synthesis
22	HSPA8, transcript variant 1	Binds to nascent polypeptides to facilitate correct folding	Protein synthesis
23	ENO1	Transcription factor activity	
24	DNAJB1	Unknown	
25	MT-ND4		
26	ZNF7		
27	UBA52		
28	Hypothetical protein BC047779	-	
29	Hypothetical protein LOC51647	-	

ACTA1, actin α 1, skeletal muscle; FHL1, four and a half LIM domains 1; EPB42, erythrocyte membrane protein band 4.2; DEFA1, Defensin α 1; ZXDC, ZXD family zinc finger C; CDK, cyclin-dependent kinase; INCA1, inhibitor of CDK interacting with cyclin A1; S100A8, S100 calcium-binding protein A8; Rho, Ras homology; PFN1, profilin 1; CEACAM8, carcinoembryonic antigen-related cell adhesion molecule 8; COL1A1, collagen, type I, α 1; COL1A2, collagen, type I, α 2; ACSL6, Acyl-Coa synthetase long-chain family member 6; FABP4, fatty acid-binding protein 4, adipocyte; GMPS, guanine monphosphate synthetase; ATP5I, ATP synthase, H^+ transporting, mitochondrial F0 complex, subunit E; MT2A, metallothionein 2A; DYNC1H1, dynein cytoplasmic 1, heavy chain 1; SVIL, supervillin; EEF1A1, eukaryotic translation elongation factor 1 α 1; RPL12, ribosomal protein L12; HSPA8, heat-shock 70 kDa protein 8; ENO1, enolase 1; DNAJB1, DnaJ (Hsp40) homolog, subfamily A, member 1; MT-ND4, mitochondrially encoded NADH dehydrogenase 4; ZNF7, zinc finger protein 7; UBA52, UBA52 ubiquitin A-52 residue ribosomal protein fusion product 1.

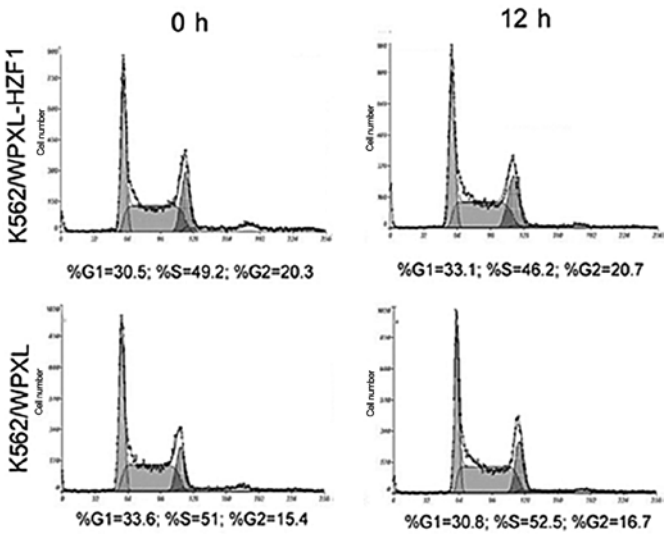

Figure 4. HZF1 overexpression promotes the S to G2/M phase transition of the K562 cell cycle. The cells of two stable K562 transductant pools were synchronized by serum starvation for 8 h, then cultured in completed medium and harvested at different time points. Cells were fixed with 75% ethanol and digested with RNaseA, then stained with propidium iodide (PI), followed by detecting the DNA content using a flow cytometer. The proportion of cells in the G2/M phase in the K562/WPXL-HZF1 cell cycle is higher than that in the K562/WPXL cell cycle, suggesting that HZF1 overexpression promotes the S to G2/M transition in the K562 cell cycle.

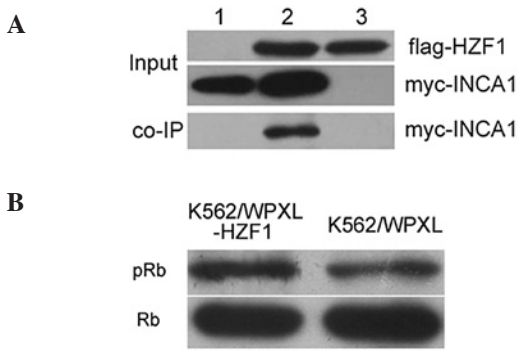

Figure 5. HZF1 interacts physically with INCA1 and inhibits its function. (A) HZF1 interacts physically with INCA1 *in vivo*. INCA1 was screened as a partner of HZF1 by Y2H. A co-immunoprecipitation (Co-IP) assay was performed to certify the interaction between HZF1 and INCA1 *in vivo*. The 293T/17 cells were co-transfected with pflag and pmyc-INCA1 (line 1), pflag-HZF1 and pmyc-INCA1 (line 2) and pflag-HZF1 and pmyc (line 3), respectively. Following a 36-h transfection, cells were harvested and lysed, and the expression of target-fused proteins was identified by Western blot analysis with anti-flag and anti-myc antibody, respectively. A Western blot analysis was then performed with the anti-myc antibody after the proteins enriched by Co-IP were denatured with the anti-flag monoclone antibody. The results showed that INCA1 is the interaction partner of HZF1 (Line 2). (B) HZF1 inhibits the function of INCA1, an inhibitor of CDK2 interacting with cyclin A1. A significantly higher level of pRb was identified in the K562/WPXL-HZF1 cells with HZF1 overexpression compared to the control K562/WPXL cells, suggesting that HZF1 inhibits the role of INCA1 and facilitates the activation of CDK2.

A, is ubiquitously expressed and is involved in the S phase and G2/M transition through its association with distinct CDKs (13,14). Cyclin A2 associates with CDK2 (15) at the onset of DNA replication in S phase (16) and with CDK1 mainly at the G2/M transition (13). The second type of human cyclin A, known as cyclin A1, associates with CDK2 *in vitro* and *in vivo,* but not with CDK1 (17). Cyclin A1 expression is mainly restricted to the testis in healthy humans (17), it is also highly expressed in leukemias of myeloid origin (18). Cyclin A1 expression increases at the entry into the S phase of previously synchronized leukemic cells (17), and cyclin A1 expression and cyclin A1-CDK2 kinase activity reach their maximum levels in the G2/M phase. Cyclins A1 (18) and A2 (16) bind to and activate CDK2 in the S phase, leading to the maintenance of phosphorylation and inhibition of the tumor suppressor protein pRb, and further promoting the DNA synthesis and entry into the G2/M phase.

INCA1 is an inhibitor of CDK2 interacting with cyclin A1. It inhibits the process of S to G2/M transition of the cell cycle. This study showed that HZF1 interacts with INCA1 *in vitro* and *in vivo* and promotes the S to G2/M transition of the K562 cell cycle, resulting in a hypothesis that HZF1 rescues the CDK2 activity by competitively inhibiting the interaction between INCA1 and the cyclin A-CDK2 complex. To verify this hypothesis, we identified the pRb level, which may reflect the activity of CDK2. A higher level of pRb was observed in K562 cells with HZF1 overexpression, while no marked expression exchange of related cyclins was identified by real-time PCR in these cells (data not shown). Our results suggested that HZF1 may competitively inhibit the INCA1 binding to cyclin A-CDK2, enhancing the CDK2 activity and promoting the cell proliferation of K562 cells.

In conclusion, HZF1 plays a role in cell cycle regulation, and may function by enhancing CDK2 activity by interaction with, and inhibition of, INCA1.

Acknowledgements

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