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Humanin delays apoptosis in K562 cells by downregulation of P38 MAP kinase

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Humanin (HN) is a newly identified neuroprotective peptide. In this study, we investigated its antiapoptotic effect and the potential mechanisms in K562 cells. Upon serum deprivation, expression of HN in K562 cells decreased and its intracellular distribution changed from cytoplasm to cell membrane. In HN stably transfected K562 cells, apoptosis was delayed compared with control vector transfected cells as measured by flow cytometry. Furthermore, analysis of different mitogen-activated protein (MAP) kinases activity revealed that extracellular signal-regulated kinase (ERK) pathway was inhibited while p38 signaling was activated following serum deprivation in K562 cells. And in HN transfected K562 cells, ERK downregulation was not affected, but p38 activation was suppressed, which may responsible for the delayed apoptosis in these cells. Activation of the ERK signaling pathway by phorbol myristate 13-acetate (PMA) and sorbitol protected K562 cells from serum deprivation induced apoptosis. Additionally, overexpression of HN reduced megakaryocytic differentiation of K562 cells. The present data outline the role of ERK and p38 MAP kinases in serum deprivation induced apoptosis in K562 cells and figure out p38 signaling pathway as molecular target for HN delaying apoptosis in K562 cells.

Keywords: apoptosis; differentiation; humanin; K562 cells; MAPK.

Introduction

Humanin (HN) is a recently described neuroprotective peptide that antagonizes neurotoxicity caused by Alzheimer's disease (AD) relevant insults, including AD-linked mutant genes, amyloid β (Aβ) peptides and anti-APP antibody.[1–3] HN can also suppress neuronal cell death induced by serum deprivation.[4] The *in vivo* effect of HN has been observed as improving the learning and memory impairment caused by scopolamine[5] or Aβ[6] in mice. In addition, HN may be involved in protecting other cell types as well, suggested by protection against Aβ-induced toxicity in human cerebrovascular smooth muscle cells.[7]

HN cDNA has a full length of 1,567bp, which contains a 75-base ORF that encodes a 24-residue peptide. The HN peptide consists of a hydrophobic core region GFSCLLLLTSEIDL flanked by a C-terminal polar region PVKRRA and an N-terminal region MAPR, which partially satisfies the criteria of signal peptide sequences. Hashimoto *et al*. found that as a signal peptide-like molecule, HN peptide is secreted from the cells through the endoplasmic reticulum-Golgi secretory pathway and acts from outside of the cells potentially through a cell-surface receptor linked to certain tyrosine kinases.[1] Ying's work identified human G protein-coupled formylpeptide receptor-like-1 (FPRL1) as a receptor shared by both HN and Aβ42 on mononuclear phagocytes and neuronal cell lines, and more importantly, FPRL1 may act as a receptor on neuronal cells through which HN protects the cells from Aβ42-elicited apoptotic signaling cascade.[8]

The antiapoptotic mechanism of HN is not fully understood. Though FPRL1 may play a role in mediating the beneficial effects of HN, further investigation is needed to fully elucidate the mechanistic basis of the interaction of HN with its target cells. Hashimoto *et al*. have shown that HN could inhibit c-Jun N-terminal kinase (JNK) mediated neuronal cell death, and thus speculated that HN may exert its neuroprotective effect by suppressing activation of JNK and the downstream pathway.[9] Using yeast two-hybrid system, Guo *et al*. screened out HN as one of the interactive proteins to Bax.[10] They found that HN prevents the translocation of Bax from cytosol to mitochondria, and then inhibits cytochrome c and other apoptogenic proteins release from mitochondria, thus prevents apoptosis. They also proved that HN could directly suppress the targeting of Bax to mitochondria without a prerequisite to secret outside the cell. Their further studies identified two BH3-only Bcl-2/Bax-family proteins, Bid[11] and Bim[12], as additional cellular targets of HN.

Mitogen-activated protein (MAP) kinase pathways have been developed to response to and integration of

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extracellular stimuli in eukaryotic cells. The extracellular signal-regulated kinase (ERK) pathway is usually activated in response to several cytokines and growth factors, and primarily mediates mitogenic and antiapoptotic signals.[13] P38 kinase pathway is activated primarily by diverse stimuli, including cellular stresses such as UV irradiation, osmotic imbalance, protein synthesis inhibitors, but also during engagement of various cytokines receptors by their ligands.[13] Its function leads to the generation of various activities, including regulation of apoptosis and cell cycle arrest, induction of cell differentiation, as well as cytokine production and inflammation.[14,15] The JNK pathway is also activated in response to stress and growth factors, and similarly, mediates signals that regulate apoptosis, cytokine production and cell cycle progression.[13,16,17]

Though CD_{34} has been used as a convenient positive selection marker for hematopoietic stem cells (HSCs) both in research and in clinic, two elegant animal models, human/sheep competitive engraft model[18] and NOD/SCID mouse transplantation model,[19] show that there is another population of HSCs which do not express lineage commitment markers nor the CD_{34} molecule. In previous work, we identified abundant expression of HN in $Lin^- CD_{34}^-$ HSCs compared with $Lin^- CD_{34}^+$ cells,[20] so in this work, using human chronic myelogenous leukemia K562 cells, we explored the antiapoptotic effect and potential mechanisms of HN in hematopoietic system.

Materials and methods

Materials

Lipofectamine 2000 and G418 were purchased from Invitrogen (Carlsbad, CA, USA). Annexin V-FITC Kit was from Bender MedSystems (San Bruno, CA, USA). SAPK/JNK Assay Kit, p44/42 MAP Kinase Assay Kit, and P38 MAP Kinase Assay Kit were purchased from Cell Signaling Technology (Beverly, MA, USA). Rabbit polyclonal antibody raised against HN was presented kindly by Dr. Ikuo Nishimoto.

Cell culture

The human leukemia cell line K562 was grown in suspension in RPMI 1640 medium supplemented with 10%(*v*/*v*) heat-inactivated FBS, 100 units/mL penicillin and 100 μg/mL streptomycin. For experiments, exponential growing K562 cells were collected and resuspended at a density of 4×10^5 cells/mL in 24-well plates. Viable cell counts were determined using trypan-blue dye exclusion test. Apoptosis was induced by serum deprivation (0.1% FBS).

Plasmids construction and transfection

The eukaryotic expression vector pcDNA3.1/HN, which contains the 75bp ORF of HN, was constructed as follows. First, the 75bp HN ORF cDNA was amplified from a K562 reverse transcripted cDNA template using the following primers: 5′-aagcttatggctccacgagggttc-3′ (sense) and 5′-ggatccttatgcccgcctcttcac-3′ (antisense). The amplified fragment was cloned into the pcDNA3.1 vector at the Hind III and BamH I sites. Then, 1 μg aliquots of the expression vector or pcDNA3.1 empty vector were transfected using Lipofectamine 2000 in 24-well plate. Transfected cells were selected in 800 μg/mL G418 selection medium, and finally a single positive colony was isolated by limited dilution. Stably transfected K562 cells was maintained in RPMI 1640 medium containing 10% FBS and 500 μg/mL G418 without penicillin or streptomycin.

Immunocytochemistry

Control and serum-free cultured K562 cells were washed twice with ice-cold PBS and cytospined onto slides for immunocytochemistry analysis. Cells were fixed with 4% paraformaldehyde/PBS for 10 min at RT and then permeated with 0.3% Triton X-100 in PBS for 15 min at RT, followed by blocking with 20% normal goat serum for 30 min at 37°C. Cells were stained with a rabbit polyclonal antibody raised against HN at a 1:50 dilution in PBS overnight at 4°C. After washing, TRITC-labeled anti-rabbit secondary antibody was used at a 1:200 dilution to detect the primary antibody. Then cells were subjected to confocal laser microscope observation.

Apoptosis analysis

Cells were collected and washed once with ice-cold PBS. Cell pellet was resuspended with 1binding buffer (10 mM Hepes/NaOH, pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$) to $2–5 \times 10^5$/mL. After incubation with Annexin V-FITC for 10 min at RT, add PI to 1 μg/mL. Labeled cells were analyzed using a Becton Dickson FACSalibur system within 30 min.

Cell cycle analysis by flow cytometry

Cells were collected and washed twice with ice-cold PBS. Cell pellet was fixed in 70% ethanol containing 3% FBS at −20°C overnight. After centrifugation at 200 g for 3 min, cells were resuspended in PBS containing 1mg/mL RNase A and incubated for 30 min at 37°C. Then PI was added to a final concentration of 50 μg/mL, and incubated

for 30 min at 4°C. Cells were immediately subjected to flow cytometric analysis.

Assay of SAPK/JNK activity

The SAPK/JNK activity was measured with SAPK/JNK Assay Kit according to the manufacturer's protocol. 210^6 Cells were lysed in 500 μL 1cell lysis buffer (20 mM Tris (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1%TritonX-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1mg/mL leupeptin) supplemented with 100 μM PMSF for 15 min on ice. 250 μL of cell lysate ($\sim$250 μg total protein) was mixed with 2 μg of GST-c-Jun fusion protein beads and incubated with gentle rocking overnight at 4°C. The lysate was centrifuged for 30 s at 4°C, and then the pellet washed twice with 500 μL of lysis buffer and twice with kinase buffer (20 mM Tris–HCl, pH 7.5, 5 mM β-glycerophosphate, 2 mM DTT, 0.1 mM Na_3VO_4 10 mM $MgCl_2$) on ice. The pellet was suspended with kinase buffer supplemented with 100 mM ATP and incubated for 30 min at 30°C. The reaction was terminated with 25mL 3sample buffer (final 1concentration; 62.5 mM Tris–HCl, pH 6.8, 2%SDS, 10%glycerol, 50 mM DTT, 0.1%bromophenol blue) and the sample was analyzed by 12%SDS-PAGE. Phospho (Ser63)-c-Jun was detected with specific antibody by western blot analysis.

Assay of ERK/MAPK and p38 kinase activity

ERK:MAPK activity was measured with p44/42 MAP Kinase Assay Kit according to the manufacturer's protocol. 210^6 Cells were lysed in 500 μL 1cell lysis buffer supplemented with 100 μM PMSF for 15 min on ice. 200 μL of cell lysate (approx. 200 μg protein) was mixed with immobilized phospho-p44/42 MAP kinase monoclonal antibody and incubated with gentle rocking overnight at 4°C. The lysate was centrifuged for 30 s at 4°C, and then the pellet washed twice with 500 μL of lysis buffer and twice with kinase buffer on ice. Then the pellet was suspended with 50 μL kinase buffer containing 200 μM ATP and 2 μg Elk1 fusion protein and incubated 30 min at 30°C. The reaction was terminated with 25 μL 3sample buffer and the sample was analyzed by 12% SDS-PAGE. Phospho (Ser383)-Elk1 was detected with specific antibody by western blot analysis.

For p38 kinase assay, the same procedure with ERK/MAPK assay was used, except that immobilized p38 MAP kinase monoclonal antibody and phospho(Thr71)-ATF-2 antibody were used instead of immobilized phospho-p44/42 MAP kinase monoclonal antibody and phospho (Ser383)-Elk1 antibody, respectively.

Western blot analysis

The above samples were subjected to SDS-PAGE using 12% SDS polyacrylamide gels and transferred to PVDF membranes using a semidry electroblot chamber. Membrane was blocked in TBS pH 7.4 containing 0.1% Tween-20 (TBST) and 5% non-fat milk for 1 h at RT. Incubation with primary antibodies were carried out at 4°C overnight at a 1:1000 dilution in TBST containing 5% BSA. Following 1 h incubation with goat-anti-rabbit peroxidase conjugated antibody at RT, protein was detected by the ECL method according to the manufacturer's instruction.

Statistical analysis

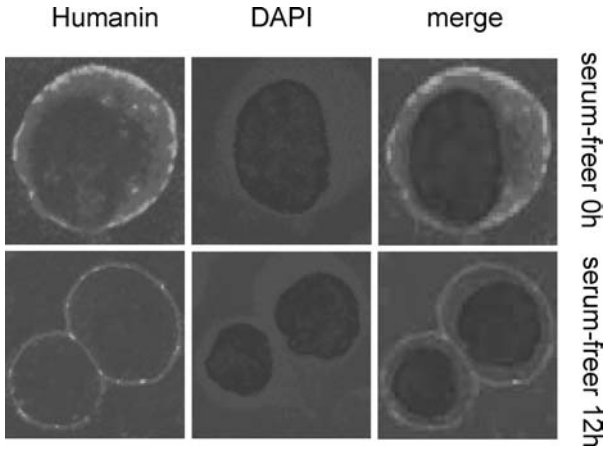
Data are presented as the mean $\pm$ SD. Statistical significance was calculated using *t* test for all analyses.

Results

Changed localization of HN in serum-free cultured K562 cells

HN is an effective neuroprotective factor. To investigate whether it may have antiapoptotic effect in hematopoietic system, its expression in K562 cells was determined. As shown in Figure 1, expression of HN in K562 cells decreased and its intracellular distribution changed from cytoplasm to cell membrane after K562 cells being cultured in serum-free medium for 12 h. The distribution alteration of HN is intriguing and indicates its involvement

Figure 1. Localization change of Humanin in K562 cells after serum deprivation. K562 cells were cultured in the presence of 0.1%FBS (serum-free) for 12 h. After harvesting, intracellular expression of Humanin was detected by immunocytochemistry. TRITC-labeling was used to indicate localization of HN, DAPI to label the nuclei. Cells were examined by confocal laser microscope.



in the serum depravation induced apoptotic process of K562 cells.

Overexpression of HN in K562 cells delayed apoptosis by serum deprivation

We then examined whether overexpression of HN affects susceptibility of K562 cells to apoptosis induced by serum deprivation. Apoptosis was measured by Annexin V and PI double staining. Results showed that control vector-transfected K562 cells were much more susceptible to serum-free induced apoptosis than HN-transfected K562 cells at the early stage, and then HN-transfected K562 cells also went apoptosis in comparative rate with control vector-transfected K562 cells (Figure 2A). Cell viability was determined by trypan blue exclusion assay. As illustrated in Figure 2B, in serum-free conditions, HN-transfected K562 cell number expanded more than 1.5-fold in 24 h, whereas control vector-transfected K562 cells showed only a subtle increase. But after 24 h, both groups of cell showed a similar decline rate in cell number. The above results suggest that HN overexpression just delays or partially protects K562 cells from apoptosis induced by serum deprivation at the early stage, but can not completely block the process.

We next examined cell cycle change using flow cytometry protocol. After 24 h culture in serum-free medium, there was a little to no cell cycle change in HN-transfected K562 cells, consistent with the above results that in 24 h, HN-transfected K562 cells showed a nearly normal expansion rate. But in control vector-transfected K562 cells, a significant G2/M arrest was observed (Figure 2C). Therefore serum deprivation interfere both cell viability and cell cycle in K562 cells, and HN may play critical roles on both processes.

Activation of p38 kinase and inhibition of ERK contribute to serum deprivation induced apoptosis in K562 cells

During the course of apoptosis induced by cellular stresses, JNK and p38 kinase signaling pathways are generally activated. Recently, more and more results show that activation of the JNK and p38 kinase and concurrent inhibition of ERK activity are critical for apoptosis.[21,22] Studies by Hashimoto *et al.* showed that HN blocked neurotoxicity by suppressing activation of JNK.[9] Therefore, to determine whether JNK, p38 and ERK are associated with serum deprivation induced apoptosis of K562 cell, and whether HN delays apoptosis through modulating the activity of these three MAP kinases, activity of JNK, p38 and ERK kinases were assayed.

JNK was not activated after serum deprivation (Figure 3A), indicates that it does not participate in this process. In contrast, p38 kinase was activated time-dependently after serum deprivation (Figure 3B), and its activity was peaked at 60 min in HN-transfected K562 cells and at 120 min in control vector-transfected K562 cells. Scanning densitometry indicates a ~1.4-fold difference of p38 activity between these two peak values. Meanwhile, the activity of ERK was markedly inhibited

Figure 2. Humanin delayed the onset of apoptosis in K562 cells. HN- and control vector-transfected K562 cells (HN-K562 and 3.1-K562) were cultured in serum-free medium for indicated time and then (A) stained with Annexin V-FITC and PI, apoptosis measured by flow cytometric analysis; (B) cell viability measured using trypan blue exclusion assay; or (C) stained with PI for cell cycle analysis. All experiments were repeated three times and standard deviation calculated. *,** $p < 0.05$.

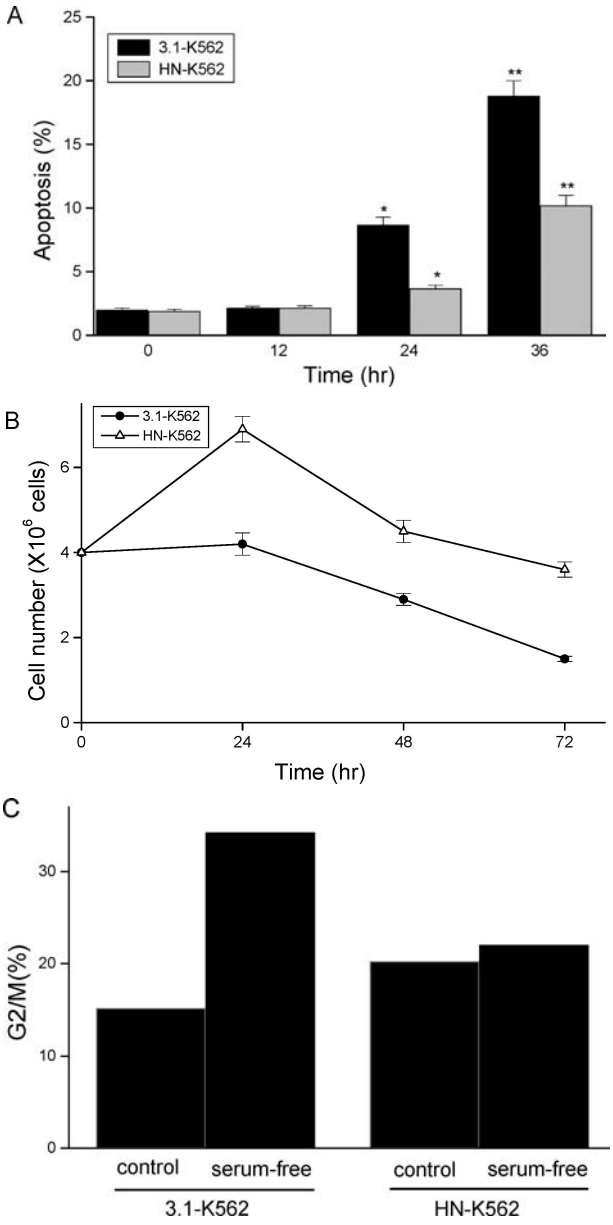
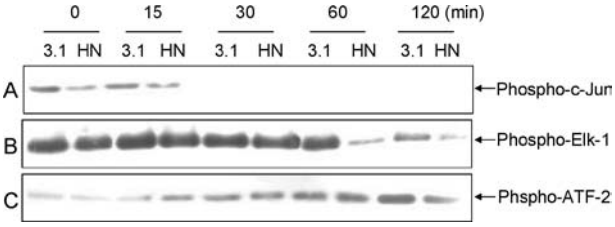


Figure 3. Effects of serum deprivation on activity of mitogen-activated protein kinases in K562 cells. HN- and control vector-transfected K562 cells (HN and 3.1) were cultured in serum-free medium for indicated time and then subjected to kinase assay. (A) JNK activity showed no apparent change. (B) Activation of p38 kinase after serum deprivation. (C) Inhibition of ERK activity by serum-free treatment.



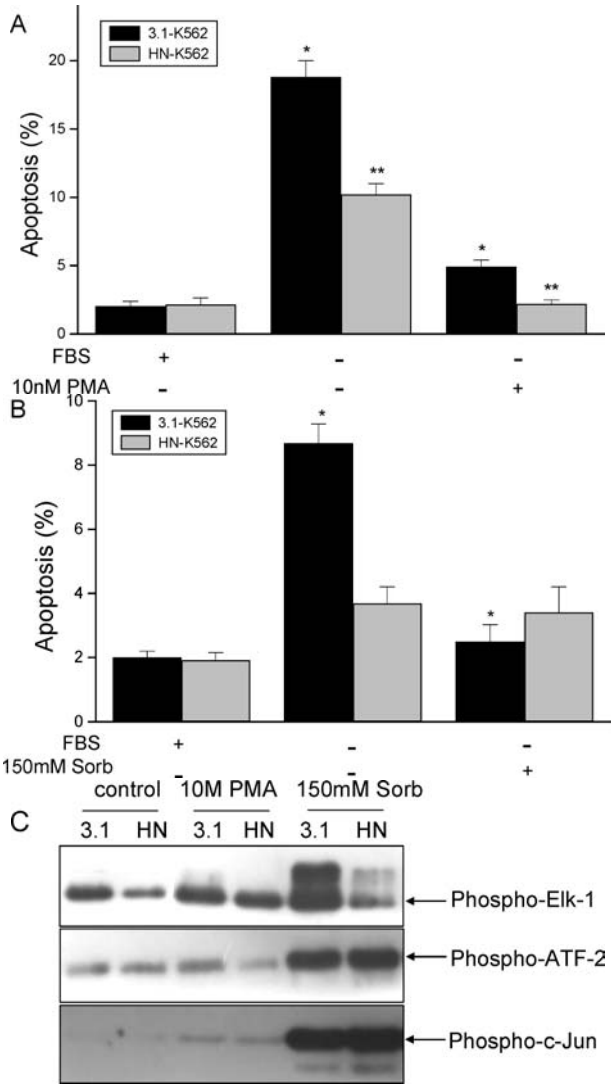
by serum-free treatment in both groups of cell (Figure 3C). However, HN-transfected K562 cells showed a rapid and enhanced decrease of the ERK activity compared with control vector-transfected K562 cells. These findings suggest that activation of p38 kinase and inhibition of ERK signaling pathway participate in serum deprivation induced apoptosis in K562 cells, and HN delays the process might through downregulation of p38 activity.

Activation of ERK signaling pathway prevented apoptosis by serum deprivation in K562 cells

Kang *et al.* have reported that inhibition of ERK, but not activation of JNK or p38 is essential for inducing apoptosis in K562 cells.[21] Therefore, we next examined the effect of phorbol myristate 13-acetate (PMA), an ERK activator on apoptosis by serum deprivation in K562 cells. As expected, pretreatment with PMA activated ERK signaling pathway and had little to no effect on the activity of JNK and p38 (Figure 4C), and eventually completely blocked the apoptosis (Figure 4A).

Sorbitol is a strong activator of JNK and p38 kinase. To determine whether HN overexpression could suppress sorbitol induced apoptosis, K562 cells were treated with sorbitol in serum-free medium. Though 75 mM sorbitol had no effect on viability of K562 cells, unexpectedly, 150 mM sorbitol treatment exhibited protective effect on serum-free cultured K562 cells but not enhancing the apoptotic effect. Compared with the protective effect of HN, 150 mM sorbitol did not abolish apoptosis in HN-transfected K562 cells though it did in control vector-transfected K562 cells (Figure 4B). MAP kinase assays revealed that sorbitol treatment activated all these 3 kinases in control vector-transfected K562 cells, but in HN-transfected K562 cells, ERK activation was suppressed (Figure 4C). These might be the cause why sorbitol could not completely block apoptosis in HN-transfected K562 cells. And the results also confirm the previous report that inhibition of ERK, but not activation of JNK or p38 is primary to induce apoptosis in K562 cells.[21]

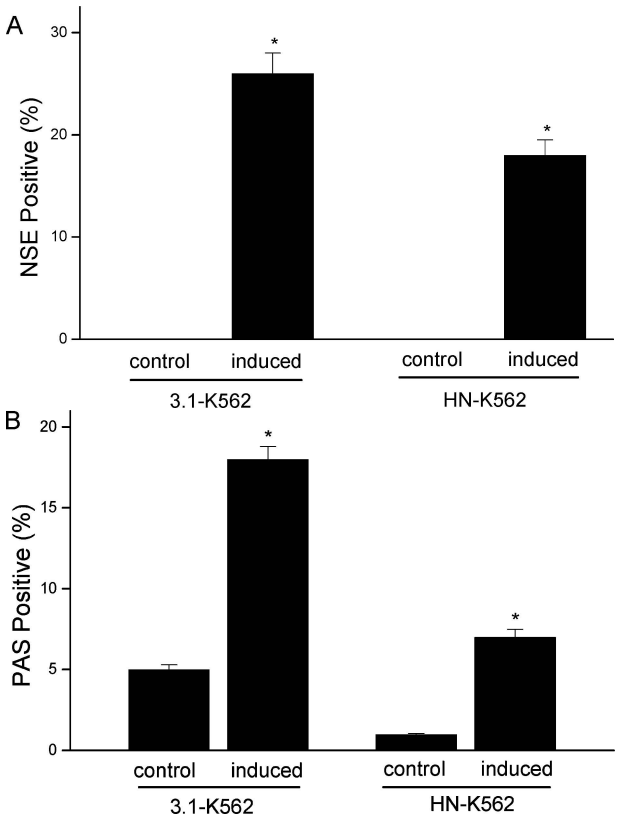
Figure 4. PMA and sorbitol treatment protected K562 cells from apoptosis through activation of ERK. (A) HN- and control vector-transfected K562 cells (HN-K562 and 3.1-K562, or HN and 3.1) were pretreated by 10 nM PMA for 30 min and then cultured in serum-free medium for 36 h for apoptotic analysis. (B) HN- and control vector-transfected K562 cells (HN-K562 and 3.1-K562, or HN and 3.1) were treated with 150 mM sorbitol in serum free medium for 24 h for apoptotic analysis. (C) After treatment with PMA or sorbitol for 30 min, kinase assay was performed as described in materials and methods. All experiments were performed three separate times. *,** $p < 0.05$. Sorb indicates sorbitol.



Overexpression of HN decreased megakaryocytic differentiation of K562 cells

Previous studies have shown that sustained activation of ERK signaling pathway is required for megakaryocytic

Figure 5. Humanin decreased megakaryocytic differentiation of K562 cells. HN- and control vector-transfected K562 cells (HN-K562 and 3.1-K562) were induced to differentiate along megakaryocytic lineage by treatment with 10 nM PMA for 72 h. Untreated cells were indicated as control. And then cells were cytospined onto glass slides for NSE and PAS staining. One hundred cells were counted each time to obtain the positive rate. Data are expressed as mean ± SD, $n = 3$. * $p < 0.05$.



differentiation of K562 cells.[23,24] From the above results, we can see that both before and after PMA treatment, HN-transfected K562 cells have a relatively lower ERK activity. To identify whether overexpression of HN would affect megakaryocytic differentiation of K562 cells, 10 nM PMA was used to induce megakaryocytic differentiation of both HN-transfected and control vector-transfected K562 cells. Megakaryocytic differentiation was determined by non-specific ester (NSE) staining and periodic acid-Schiff (PAS) reaction. As shown in Figure 5, overexpression of HN in K562 cells decreased its differentiation to megakaryocytic lineage.

Discussion

Humanin (HN) is a recently described neuroprotective peptide. Its cDNA was cloned from a cDNA library of the occipital lobe of an AD brain by a functional screening.[1] Whatever it is expressed intracellularly or added extracellularly as a peptide, HN is able to suppress cell death induced by all kinds of AD-related insults in neuronal cells, such as toxic Aβ peptides (Aβb25–35 and Aβ1–42/43), anti-APP antibody and familial AD (FAD)-causative mutants of AβPP, presenilin (PS)-1 and PS2.[1–3] Though HN does not demonstrate any protective effects on neuronal cells subjected to polyglutamine repeat (Q79), superoxide dismutase-1 mutations or etoposide treatments, subsequent studies have demonstrated that HN could inhibit serum deprivation induced cell death in PC12 cells,[4] and rescue cortical neurons from the soluble prion peptide PrP(118–135) induced apoptosis.[25] These observations suggested that HN could have a broader spectrum of protection against cell death-inducing stimuli other than AD-related insults.

In the present paper, we examined whether HN has antiapoptotic effect in K562 cells. Preliminary results showed that, after culturing in serum-free medium for 12 h, expression of HN in K562 cells decreased and its distribution changed from cytoplasm to cell membrane. Hashimoto *et al*. found that as a signal peptide-like sequence, HN must be secreted outside the cell to exert its antiapoptotic effect, and there may be a specific binding site on the cell surface for it.[1] Therefore, changed distribution of HN to cell membrane in serum-free cultured K562 cells indicates its involvement in the apoptotic process.

Then HN was ectopically expressed in K562 cell to examine its antiapoptotic effect. Using Annexin V and PI double staining method, we found that HN delayed the onset of apoptosis, but after culturing in serum-free medium for 36 h, HN-transfected K562 cells also went apoptosis in comparative rate with control vector-transfected cells. These results are different from previous findings using neuronal cells *in vitro*, which showed that usually more than 95% viability could be obtained once HN was added together with the insults,[1–3] and suggest HN might use distinct antiapoptotic signaling pathways in different cell types. Recently, the human G protein-coupled FPRL1 was identified as a receptor on neuronal cells through which HN protects the cells from Aβ42-elicited apoptosis.[8] Whether HN acts on the same receptor in non-neuronal cells require further investigation.

In response to a range of stresses, including DNA damage, hypoxia or proliferative signals, cells used to undergo either cell cycle arrest or apoptosis.[26] The response to cell cycle arrest is a checkpoint function that allows cells to pause in the cell cycle either temporarily or permanently to prevent the perpetuation of potentially oncogenic mutations. The apoptotic response is one that acts to cull cells that are proliferating aberrantly or that have suffered DNA damage, such as through checkpoint or repair defects. These two processes are not always independent; some researches have shown that the

G2/M phase-arrested cells are more sensitive to apoptotic stimulation.[27,28] In this experiment, we observed that serum deprivation caused a significant G2/M arrest in control vector-transfected K562 cells, while HN overexpression compromised the effect. So we speculated that HN might affect the cell cycle arrest, that is, decrease the cell number retarded at the G2/M phase, and then protect the cells from apoptosis.

MAP kinase pathways have critical roles in cellular responses to various extracellular signals. The ERK pathway activation is usually associated with cell growth both in nervous[29] and hematopoietic[30] systems and its downregulation usually leads to cell cycle arrest and/or apoptosis. For example, Fucoidan, a sulfated polysaccharide in brown seaweed, was found to inhibit proliferation and induce apoptosis through downregulation of ERK in human lymphoma HS-Sultan cell lines.[31] Treat primary acute myelogenous leukemia blasts with PD98059, a selective inhibitor of MEK1 phosphorylation, induced a G1-phase accumulation followed by the apoptosis of a significant percentage of the leukemic blasts.[32] However, activation of JNK and p38 kinase pathways usually leads to cell apoptosis.[33,34] An experiment using a JNK-specific inhibitor SP600125 or a p38 MAP kinase inhibitor SB202190 indicated that benzyl isothiocyanate (BITC)-induced apoptosis might be regulated by the activation of these two kinases.[27] While BITC is likely to confine the Jurkat cells in the G2/M phase mainly through the p38 MAP kinase pathway.[27] Since studies by Hashimoto *et al.* have shown that HN blocked neurotoxicity by suppressing activation of JNK.[9] In the current report, we examined the three MAP kinases' activity in K562 cells after serum deprivation, and found that inhibition of ERK and activation of p38 kinase but not JNK pathway are associated with the cell cycle arrest and apoptotic process. Since intracellularly expressed HN only interferes with activation of p38 but not downregulation of ERK, the p38 signaling pathway is supposed to be involved in both cell cycle arrest and apoptosis in K562 cells after serum deprivation. These results suggest that HN might have multiple targets to complete its antiapoptotic effect.

Since PMA pretreatment and sorbitol treatment protected K562 cells from apoptosis by serum deprivation, suppression of ERK activity may play a key role in this process. This supported the conclusion that inhibition of ERK, but not activation of JNK or p38 is primarily required to induce apoptosis in K562 cells.[21] This also explained why HN just delays the apoptotic process, but cannot completely abolish apoptosis in this study.

The K562 cells undergo megakaryocytic differentiation in response to PMA stimulation. This event correlates with MAP kinase activation and cell cycle arrest. Several previous studies using a variety of experimental systems have reinforced the central role of sustained ERK activation in the programming of megakaryocytic lineage commitment.[23,24] Shelly *et al.* have isolated K562 cells that are resistant to the growth inhibitory action of PMA.[35] Molecular characterization demonstrates that PMA resistance is downstream from PMA induced activation of the ERK pathway. Consistent with previous findings, our results showed that suppression of ERK activity by overexpression of HN decreased megakaryocytic differentiation of K562 cells.

HSCs are of interest both in clinical medicine and in basic developmental biology.[36] They could be used for rescuing the patient from the effects of high doses of chemotherapy or used as a target for gene-therapy vectors. In parallel with these clinical utility, there has been much interest in elucidating the molecular mechanisms by which HSC replication and differentiation are regulated. A better understanding of these mechanisms is of interest from the perspective of basic science and for developing new approaches for HSC transplantation. When HSCs divide, one possible outcome is the generation of new HSCs—a process known as self-renewal. Alternatively, HSCs can differentiate into more mature blood cells or can be lost by apoptosis. So to self-renew means that it cannot go apoptosis or differentiation, thus a stem cell need at least two signals to go self-renew: one for expansion, another for not go apoptosis or differentiation.[37] Present data from K562, a human erythroleukemic progenitor cell, showed that HN not only delayed apoptosis in this cell but also decreased its differentiation to megakaryocytic lineage. Since HN was found to be abundantly expressed in $Lin^{-}CD34^{-}$ HSCs [20], we proposed that HN might supply the later signal to HSCs and be involved in the maintenance of HSC reservoir *in vivo*.

Conclusion

This study identified Humanin, a neuroprotective factor, could also protect K562, a human erythroleukemic progenitor cell, from apoptosis induced by serum deprivation. This observation further strengthened the broad spectrum of the protective effect of HN. P38 MAP kinase is involved in serum deprivation induced cell cycle arrest and apoptosis, and HN blocks both processes through suppressing p38 activation.

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