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Stem cell factor (SCF) protects osteoblasts from oxidative stress through activating c-Kit-Akt signaling

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ABSTRACT

Osteoblasts regulate bone formation and remodeling, and are main target cells of oxidative stress in the progression of osteonecrosis. The stem cell factor (SCF)-c-Kit pathway plays important roles in the proliferation, differentiation and survival in a range of cell types, but little is known about its functions in osteoblasts. In this study, we found that c-Kit is functionally expressed in both osteoblastic-like MC3T3-E1 cells and primary murine osteoblasts. Its ligand SCF exerted significant cyto-protective effects against hydrogen peroxide (H_2O_2). SCF activated its receptor c-Kit in osteoblasts, which was required for its cyto-protective effects against H_2O_2. Pharmacological inhibition (by Imatinib and Dasatinib) or shRNA-mediated knockdown of c-Kit thus inhibited SCF-mediated osteoblast protection. Further investigations showed that protection by SCF against H_2O_2 was mediated via activation of c-Kit-dependent Akt pathway. Inhibition of Akt activation, through pharmacological or genetic means, suppressed SCF-mediated anti-H_2O_2 activity in osteoblasts. In summary, we have identified a new SCF-c-Kit-Akt physiologic pathway that protects osteoblasts from H_2O_2-induced damages, and might minimize the risk of osteonecrosis caused by oxidative stress.

1. Introduction

Osteoblasts regulate bone formation and remodeling, and are the main target cells of oxidative stress in the progression of osteonecrosis. The level of reactive oxygen species (ROS), including superoxide, hydroxyl radical, singlet oxygen and hydrogen peroxide (H_2O_2), is increased in osteonecrosis tissues, causing damages to osteoblasts and other bone cells, which serves as an important pathological mechanism of osteonecrosis [1,2]. Recently, different groups have been adding H_2O_2 to cultured osteoblasts to create cellular models of osteonecrosis [3,4].

Signaling of stem cell factor (SCF) and its receptor c-Kit (or CD-117) [5,6] plays a vital role in normal hematopoiesis, melanogenesis, and gametogenesis [7]. Besides functioning as a transcriptional factor in regulation of development processes, SCF binding to c-kit can trigger pathways involved in cell survival and other important cellular functions [8,9]. For example, SCF treatment induces activation of phosphatidylinositol-3-kinase (PI3K)-Akt signaling in a number of established cell lines to promote cell survival [8,9]. Little is known, however, about SCF/c-Kit signaling and its functions in osteoblasts.

Here, we studied the possible cyto-protective effect of SCF/c-Kit against H_2O_2-induced oxidative stress in cultured osteoblasts. We identified a new SCF/c-Kit-mediated pro-survival pathway that protects osteoblasts from H_2O_2 damages.

2. Materials and methods

2.1. Chemicals and antibodies

H_2O_2, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) formazan and LY294002 were purchased from Sigma Chemical Co. (St. Louis, MO). Perifosine, PD98059, NVP-BEZ235, STI-571 and BMS-354825 were obtained from Selleck Chemicals LLC (Houston, TX). Antibodies against phospho(p)-Akt (Ser-473), p-Akt (Thr-308), Akt, p-c-Kit (Tyr-719), c-Kit and tubulin were purchased from Cell Signaling Technology (Denver, MA).

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2.2. MC3T3-E1 cell culture

Murine osteoblastic MC3T3-E1 cells, purchased from Shanghai Institute of Biological Science (Shanghai, China), were maintained in α-MEM medium, supplemented with 10% fetal bovine serum (FBS, Gibco-BRL, Grand Island, NY), 1% penicillin/streptomycin (Gibco), in a humidified atmosphere of 5% CO_2 at 37 °C. Cultures were trypsinized upon confluence and sub-cultured into 12-, 6-, or 96-well plates for further experiments.

2.3. Primary osteoblasts isolation and culture

As reported [10], murine osteoblasts were isolated from pups at 30–36 h old. The animals were sedated by hypothermia and then killed by decapitation. The calvariae were removed and bathed in α-MEM. The fibrous tissue surrounding the bone was gently scraped, and sutures were removed. The trimmed calvariae were transferred to a 50-mL Erlenmeyer flask and washed with phosphate-buffered saline (PBS, Gibco) three times. Calvariae were then subjected to a series of collagenase-I (Sigma) digestions in an oscillating 37 °C water bath. The first two digests were discarded. Digests 3–5 were neutralized with α-MEM with 10% FBS, pooled, and filtered through sterile polypropylene mesh. The single cell suspension were resuspended in 3–5 mL α-MEM containing 20% FBS with antibiotics. Cells were counted and cultured until reaching confluence, half of the medium was renewed every 2 days.

2.4. MTT cell viability assay

Osteoblast cell viability was detected by MTT assay. Briefly, cells were plated at 6×10^3 cells/well in a 96-well plate with 60% confluence. After treatment, 10 μL of MTT solution (Sigma, 1 mg/mL) was added to each well, and the cells were incubated for another 3 h at 37 °C. After formation of formazan crystals, the medium was then aspirated very carefully and replaced with 150 μL of dimethyl sulfoxide (DMSO, Sigma) for dissolving the formazan crystals, and the plates were shaken for 5 min. The absorbance of each well was recorded on a microplate spectrophotometer at 490 nm. The optical densities (OD) value was utilized as a meter of cell viability.

2.5. Evaluation of apoptotic cell death by ELISA

Analysis of DNA fragmentation was performed with the cell death detection enzyme-linked immunosorbent assay (ELISA) kit based on the photometric sandwich immunoassay of cytoplasmic histone associated DNA fragments (Roche Molecular Biochemicals, Indianapolis, IN), using manufacturer's recommended protocols. After addition of substrate for peroxidase, the spectrophotometric absorbance of the samples was determined using a plate reader at a test wavelength of 405 nm. OD value was utilized to reflect cell apoptosis.

2.6. Caspase-3 activity assay

Caspase-3 activity was determined using the Apo-ONE homogeneous caspase-3 activity kit (Promega, Madison, WI), according to manufacturer's instructions. In this system, the caspase-3 substrate rhodamine 110, bis-(N-CBZ-L-aspartyl-L-glutamyl-L-valyl-L-aspartic acid amide (Z-DEVD-R110), exists as a pro-fluorescent substrate prior to the assay. Addition of active caspase-3 from cellular lysates results in the cleavage of the DEVD peptides and following excitation at 500 nm, the rhodamine 110 leaving group becomes intensely fluorescent. The amount of fluorescent product generated is proportional to the amount of caspase-3 cleavage activity present in the sample. Samples and substrates were maintained in a water bath at 37 °C for 45 min and then analyzed in a fluorescent spectrophotometer at 500 nm. Relative fluorescent units (in OD) was recorded as the indicator of caspase-3 activity.

2.7. LDH assay of necrosis

Cell necrosis was assessed by the release of lactate dehydrogenase (LDH) into the culture medium, which indicates loss of membrane integrity (cell necrosis). LDH activity was measured using a commercial kit (Kyokuto Chemical Co., Tokyo, Japan). Percent of cell necrosis was expressed as: medium LDH value/(medium LDH value plus cell lysates LDH value) × 100%, cell lysates were obtained by incubating cells in 0.2% Triton X-100 for 15 min at 37 °C.

2.8. Western blots

After treatments, cells were washed twice with cold PBS and resuspended in five volumes of ice-cold extract buffer (20 mM Western blotting analysis Hepes–KOH, 1.5 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 1 mM DTT and 0.1 mM phenylmethanesulfonyl fluoride (PMSF), pH 7.5 for 15 min at 4 °C. The samples (10 μg proteins) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) with 10% polyacrylamide gel, proteins were then electrotransferred to a polyvinylidenefluoride (PVDF) membrane, which was then washed in a blocking solution [5% nonfat milk in TBST buffer (20 mM Tris–HCl, pH 7.5, 0.5 M NaCl, 0.1% Tween 20)] for 2 h at room temperature. The blots were then incubated with primary antibodies at 4 °C overnight. The appropriate secondary antibodies conjugated to horseradish peroxidase (HRP) (Santa Cruz Biotech) were then added. Antigen–antibody complex was detected by enhanced chemiluminescence (ECL) reagent. Band quantification was performed though Image J software (NIH).

2.9. RNA isolation and RT-PCR

Total RNA was extracted through TRIzol reagents (Invitrogen, Carlsbad, CA). One microgram of total RNA was reverse-transcribed (RT) using TOYOBO ReverTra Ace-a RT-PCR kit (TOYOBO, Japan) according to the manufacturer's instructions. For PCR amplification, specific oligonucleotide primer pairs (0.5 μM each) were incubated with 5 μL of cDNA template in a 50-μL PCR reaction mixture containing 1.5 mM $MgCl_2$, 25 mM KCl, 10 mM Tris, pH 9.2, 1 μL of deoxynucleotides (1 mM), and 1 unit of *Taq* polymerase. The 5′-primer for mouse c-kit was CTGGTGGTTCAGAGTTCCATAGAC. The 3′ primer for c-kit was TCAACGACCTTCCCGAAGGCACCA. Dilutions of cDNA were amplified for 26–28 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s. The amplified PCR products were analyzed by 5% polyacrylamide gel electrophoresis and ethidium bromide staining.

2.10. ShRNA and stable cell selection

ShRNAs specifically targeting mouse Akt1 (Santa Cruz, sc-29196-SH) or mouse c-Kit (Santa Cruz, sc-29852-SH), and the non-target scramble control shRNA (Santa Cruz, sc-108060) were commercially obtained. All sequences were confirmed by commercial sequencing. Lentiviral particles expressing shRNAs were produced in HEK-293T cells with plasmids encoding viral packaging proteins VSVG and Hit-60 (Promega) [11]. To establish stable knockdown, osteoblasts were infected with lentiviral particles at a multiplicity of infection (MOI) of 10 in all experiments. Cells were maintained in puromycin (0.5 mg/L) containing medium to establish stable cells. For each assay, at least two stable clones were picked. ShRNA efficiency was confirmed by Western blots.

2.11. Statistics

The values given were means ± standard deviation (SD) and analyzed with one-way ANOVA. The difference was significant if the *P* value was <0.05.

3. Results

3.1. Stem cell factor (SCF) protects MC3T3-E1 cells from H_2O_2

First, we examined the expression of c-Kit, the SCF receptor, in osteoblastic MC3T3-E1 cells. Western blot and RT-PCR results in Fig. 1A demonstrated that c-Kit mRNA and protein were both expressed in MC3T3-E1 cells. For detection of c-Kit protein expression, mAb against c-Kit was utilized (Cell Signaling Tech, sc-3074), and the c-Kit blot showed characteristic double bands (145/120 kDa). For RT-PCR assay, primers and size of c-Kit were same as reported [12]. MC3T3-E1 cells were then treated with varying concentrations of SCF, and cultured for 24 h, MTT cell viability assay results demonstrated that SCF alone showed no effect on cell viability except at a relative high dose (100 ng/mL), which only slightly promoted MC3T3-E1 cell proliferation (Fig. 1B). As expected, H_2O_2 treatment inhibited MC3T3-E1 cell survival, evidenced by MTT OD reduction, which was significantly inhibited by pre-treatment of SCF (10–50 ng/mL) (Fig. 1C). SCF at concentration of 50 ng/mL showed highest efficiency (Fig. 1C), inhibited MC3T3-E1 cell oxidative stress damages induced by varying concentrations of H_2O_2 (Fig. 1D). Notably, pre-treatment with SCF in MC3T3-E1 cell also inhibited H_2O_2-induced necrosis, or LDH release into the conditional medium (Fig. 1E), and cell apoptosis (Fig. 1F and G). The latter was detected by caspase-3 activity assay (Fig. 1F) and apoptosis ELISA assay (Fig. 1G). Together, these results show that c-Kit is expressed in MC3T3-E1 cells, and its ligand SCF protects MC3T3-E1 cells from H_2O_2.

3.2. SCF inhibits H_2O_2-induced damages in primary murine osteoblasts

The *in vitro* effects of SCF were also tested in primary murine osteoblasts. As shown in Fig. 2A, c-Kit mRNA and protein were again expressed in primary murine osteoblasts. Similar to the results obtained from MC3T3-E1 cells, pre-treatment of SCF suppressed H_2O_2-induced viability reduction in primary osteoblasts (Fig. 2B). Meanwhile, through the LDH assay and caspase-3 activity, we showed that induction of necrosis and apoptosis by H_2O_2 in primary osteoblasts was inhibited by SCF (50 ng/mL) pre-treatment (Fig. 2C and D). These *in vitro* findings further confirmed the cyto-protective effect of SCF against oxidative stress in osteoblasts.

3.3. SCF activation of c-Kit in osteoblasts, required for its cyto-protective effects against H_2O_2

Above results showed that c-Kit was expressed in MC3T3-E1 cells and primary osteoblasts, where its ligand SCF exerted cyto-protective effects against H_2O_2. Western blot assays in MC3T3-E1 cells showed that SCF activated c-Kit, evidenced by increased phosphorylation of c-Kit at Tyr-719 (Fig. 3A). C-Kit phosphorylation by SCF was blocked by c-Kit inhibitor Imatinib (STI-571, STI) [13] or Dasatinib (BMS-354825, BMS) [14] (Fig. 3A). Significantly, SCF-mediated cyto-protective effects against H_2O_2 were almost abolished by the two c-Kit inhibitors (Fig. 3B and C), indicating that

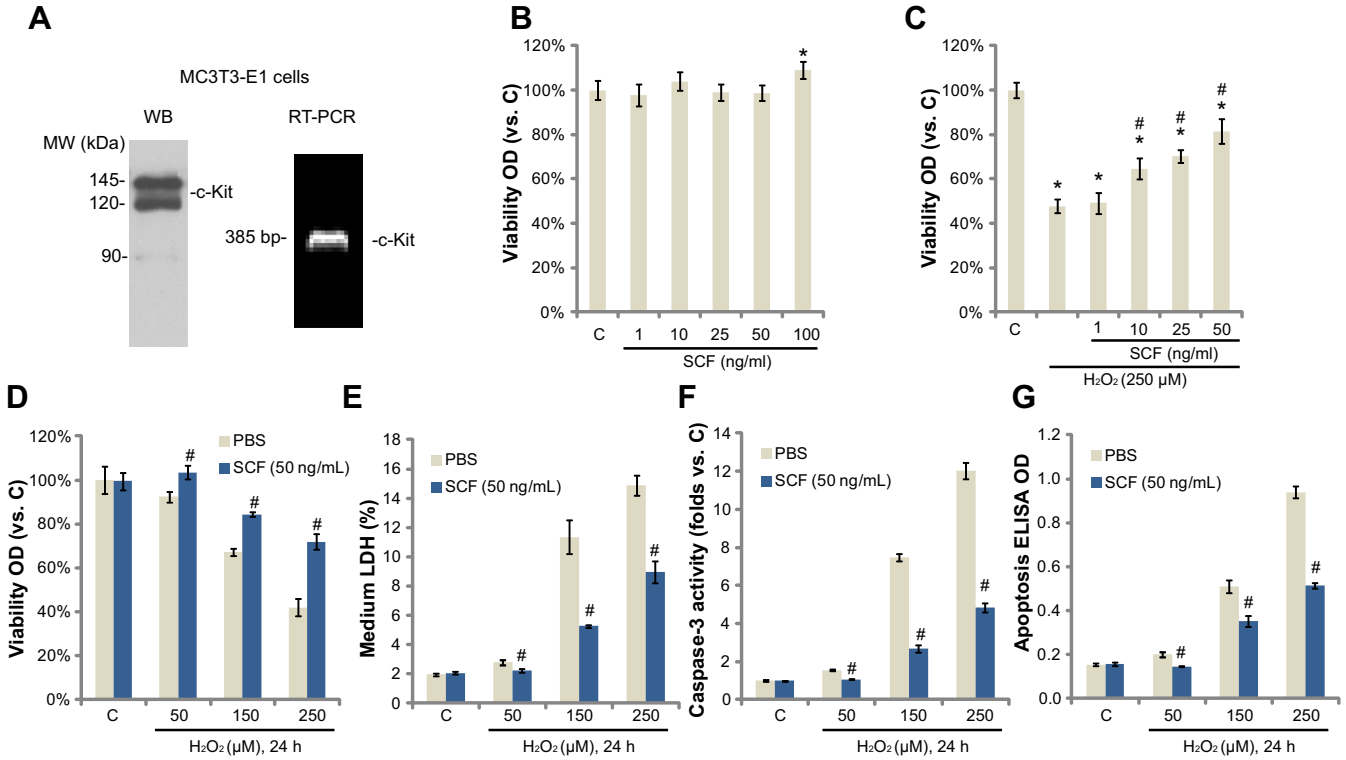

Fig. 1. Stem cell factor (SCF) protects MC3T3-E1 cells from H_2O_2. (A) Protein and mRNA expressions of c-Kit in MC3T3-E1 cells were tested by Western blot and RT-PCR, respectively. (B) MTT viability of MC3T3-E1 cells treated with indicated concentration of SCF for 24 h. MC3T3-E1 cells were pre-added with indicated concentration of SCF for 30 min, followed by H_2O_2 (50–250 μM) stimulation, cells were further cultured for 24 h, cell viability (C and D) was tested by MTT assay; cell necrosis was detected through LDH release assay (E), and cell apoptosis was tested by caspase-3 activity assay (F) and apoptosis ELISA assay (G). For each assay, $n = 6$. Each determination was done in triplicate. $^{*}P < 0.05$ vs. group "C" (control group) (B and C). $^{\#}P < 0.05$ vs. group of H_2O_2 (D–G).

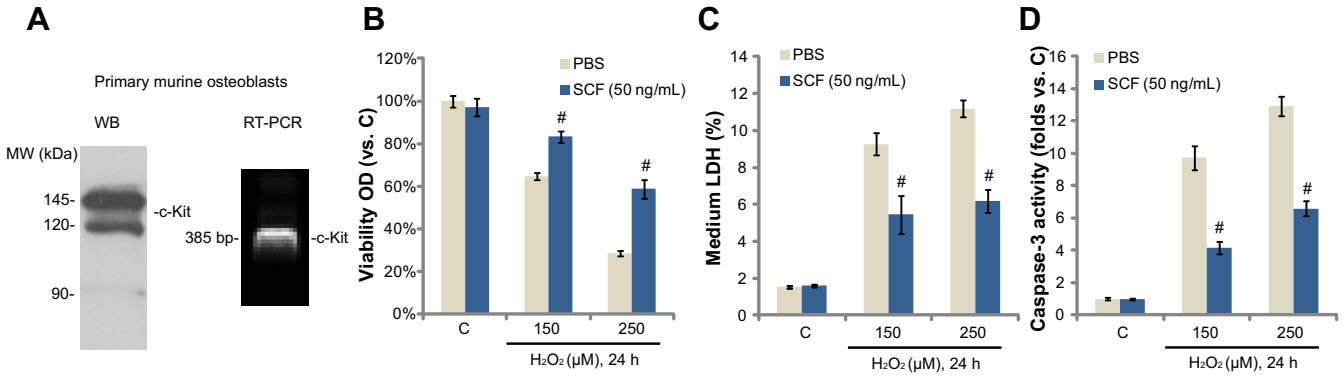

Fig. 2. SCF inhibits H_2O_2-induced damages in primary murine osteoblasts. (A) Protein and mRNA expressions of c-Kit in primary murine osteoblasts were tested by Western blot and RT-PCR, respectively. Primary murine osteoblasts were pre-added with SCF (50 ng/mL) for 30 min, followed by H_2O_2 (150–250 μM) stimulation, cells were further cultured for 24 h, cell viability, cell necrosis and cell apoptosis were tested by MTT assay (B), medium LDH release assay (C) and caspase-3 activity assay (D), respectively. For each assay, $n = 6$. Each determination was done in triplicate. $^{\#}P < 0.05$ vs. group of H_2O_2 (B–D).

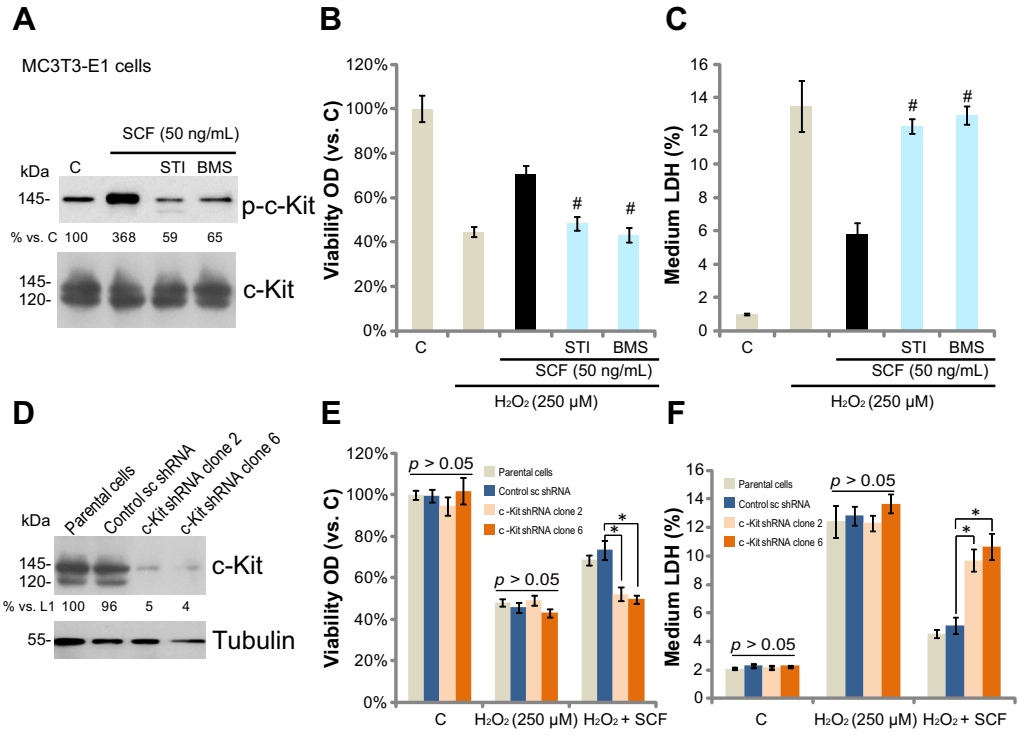

Fig. 3. SCF activation of c-Kit in osteoblasts, required for its cyto-protective effects against H_2O_2. MC3T3-E1 cells were pre-added with c-Kit inhibitor STI-571 (STI, 100 nM) or BMS-354825 (BSI, 100 nM) for 30 min, followed by SCF (50 ng/mL) stimulation for 5 min, p-c-Kit and c-Kit were tested by Western blots (A). MC3T3-E1 cells, pre-added with STI-571 (STI, 100 nM), or BMS-354825 (BSI, 100 nM) for 30 min, were treated SCF (50 ng/mL) for 30 min, followed by H_2O_2 (250 μM) stimulation, cells were further cultured for 24 h, cell viability and cell necrosis were respectively tested by MTT assay (B) and LDH assay (C). Stable MC3T3-E1 cells expressing c-Kit shRNA (clone-2/-6) or scramble control shRNA (sc shRNA), as well as the parental MC3T3-E1 cells were treated with H_2O_2 (250 μM), or plus SCF (50 ng/mL, 30 min pretreatment), expressions of c-Kit and tubulin were tested by Western blots (D), cell viability (E) and cell necrosis (F) were tested 24 h after H_2O_2 stimulation. C-Kit phosphorylation in (A) and c-Kit expression in (D) were quantified. For each assay, $n = 6$. Each determination was done in triplicate. $^{\#}P < 0.05$ vs. group of SCF only (B and C).$^{*}P < 0.05$ (E and F).

c-Kit activation is required for SCF-mediated cyto-protective effects. To rule out the possible off-target effects of these inhibitors, shRNA method was applied to selectively knockdown c-Kit. Stable MC3T3-E1 cells expressing c-Kit shRNA were selected by puromycin. Western blot results showed that c-Kit expression was dramatically downregulated in stable MC3T3-E1 cell clone-2 and clone-6 (Fig. 3D). Notably, SCF-mediated osteoblast-protective effect against H_2O_2 was dramatically inhibited in c-Kit-silenced MC3T3-E1 cells (Fig. 3E and F). Together, these results show that c-Kit activation is required for SCF-mediated cyto-protective effects in MC3T3-E1 cells.

3.4. c-Kit-dependent Akt activation mediates SCF-regulated cyto-protective effects in osteoblasts

At last we explored the signaling mechanisms of SCF/c-Kit-mediated cytoprotective effects. Akt is a major pro-survival signaling [15]. Western blot results in Fig. 4A showed that SCF activated Akt in MC3T3-E1 cells, both Akt Ser-473 and Thr-308 phosphorylations were increased after SCF stimulation, which were blocked by c-Kit inhibitor STI-571 or BMS-354825 (Fig. 4A). Significantly, SCF-regulated cyto-protective effect against H_2O_2 was inhibited by Akt inhibitors, including the pan PI3K/Akt inhibitor LY294002, Akt

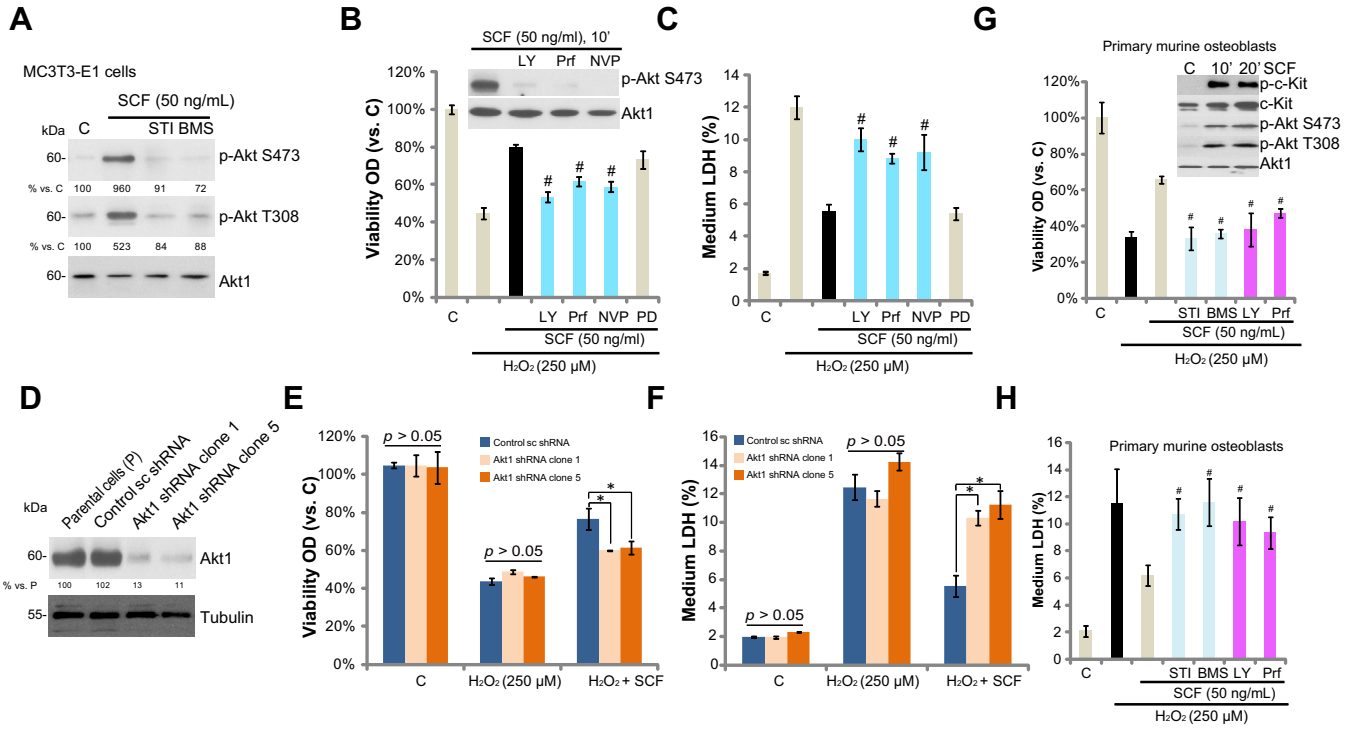

Fig. 4. c-Kit-dependent Akt activation mediates SCF-regulated cyto-protective effects in osteoblasts. MC3T3-E1 cells were pre-added with c-Kit inhibitor STI-571 (STI, 100 nM) or BMS-354825 (BSI, 100 nM) for 30 min, followed by SCF (50 ng/mL) stimulation for 10 min, p-Akt and regular Akt1 were tested (A). MC3T3-E1 cells, pre-added with LY294002 (LY, 200 nM), perifosine (Prf, 1 μM), NVP-BEZ235 (NVP, 200 nM) or PD98059 (PD, 1 μM) for 30 min, were treated SCF (50 ng/mL) for 30 min, followed by H_2O_2 (250 μM) stimulation, cells were further cultured for 24 h, cell viability and cell necrosis were tested by MTT assay (B) and LDH assay (C), respectively. Effects of these inhibitors (30 min pretreatment) on SCF (50 ng/mL, 10 min)-induced Akt Ser 473 phosphorylation was also shown (B, upper panel). Stable MC3T3-E1 cells expressing Akt1 shRNA (clone-1/-5) or scramble control shRNA (sc shRNA), were treated with H_2O_2 (250 μM), or plus SCF (50 ng/mL, 30 min pretreatment), expressions of Akt1 and tubulin were tested by Western blots (D), cell viability (E) and cell necrosis (F) were tested after 24 h. The effect of STI-571 (STI, 100 nM), BMS-354825 (BSI, 100 nM), LY294002 (LY, 200 nM) or perifosine (Prf, 1 μM) on SCF (50 ng/mL)-induced cyto-protection against H_2O_2 (250 μM) in primary murine osteoblasts was tested similarly (G and H), SCF (50 ng/mL)-induced c-Kit and Akt activation was shown in G (upper panel). Akt phosphorylation in (A) and Akt1 expression in (D) were quantified. For each assay, $n = 6$. Each determination was done in triplicate. $^{\#}P < 0.05$ vs. group of H_2O_2 only (B, C, G and H). $^{*}P < 0.05$ (E and F).

specific inhibitor perifosine and PI3K/mTOR dual inhibitor NVP-BEZ235 (Fig. 4B and C), all the inhibitors expectably blocked Akt phosphorylation by SCF (Fig. 4B, upper panel). It should be noted that Erk/MAPK was also activated by SCF-c-Kit in MC3T3-E1 cells (Data not shown), however, Erk inhibitor PD98059 showed almost no effect on SCF-regulated osteoblast protection (Fig. 4B and C).

ShRNA method was again applied to rule out possible off-target effects of the inhibitors. Stable clones of MC3T3-E1 cells were generated through infecting the lentiviral shRNA construct encoding Akt1. The Akt1 shRNA led to dramatic knockdown (over 85%) of Akt1 (Fig. 4D). In consistent with above results, Akt1 silencing significantly inhibited the cyto-protective effect of SCF against H_2O_2 in MC3T3-E1 cells (Fig. 4E and F). In primary murine osteoblasts, SCF-induced anti-H_2O_2 activity was dramatically inhibited by c-Kit inhibitors (STI-571 and BMS-354825) and PI3K/Akt inhibitor (LY294002 and perifosine) (Fig. 4G and H), both c-Kit and Akt were activated by SCF in primary murine osteoblasts (Fig. 4G, upper panel). Together, these results show that c-Kit-regulated Akt activation is required for SCF-mediated osteoblast-protective effects.

4. Discussion

In the current study, we found that c-Kit was functionally expressed in osteoblastic MC3T3-E1 cells and primary murine osteoblasts. Its ligand SCF exerted significant cyto-protective effects in osteoblasts, and suppressed H_2O_2-induced apoptosis and necrosis. SCF treatment in osteoblasts activated c-Kit-Akt signaling, which was required for its anti-H_2O_2 effect. Inhibition of c-Kit-Akt pathway, either through pharmacological inhibitors or genetic silencing, inhibited the cyto-protective effects of SCF in osteoblasts.

c-Kit is a receptor tyrosine kinase mainly expressed on the surface of hematopoietic stem cells. It is also expressed in many other cell types [7]. When binding to SCF, it forms a dimer activating its intrinsic tyrosine kinase activity, which in turn phosphorylates and activates signal transduction cascades, including the ERK-MAPK signaling, PI3K-Akt signaling, Src kinase, and JAK/STAT pathways [7]. Activation of these signaling pathways regulates cell survival, proliferation, and differentiation [7]. Akt regulates the activity of several pathways known to be involved in the control of cell survival [15]. Blume-Jensen et al. showed that SCF-mediated cell survival is through activation of PI3K-Akt-mediated phosphorylation/in-activation of Bad [8], a pro-apoptosis protein. Moller et al. found that SCF-induced mast cell survival requires activation of PI3K-Akt-mediated FOXO3a phosphorylation/inactivation [16]. In this study, we provided evidences to support that Akt activation is the major mediator of SCF/c-Kit signaling to regulate its cyto-protective effects against oxidative stress in osteoblasts. In-activation of Akt, through pharmacological inhibitors or genetic silencing, thus restrained the pro-survival effect of SCF in osteoblasts.

Akt is activated by phosphorylation at Ser-473 and/or Thr-308 [17]. In the current study, we observed that Akt became rapidly

phosphorylated at both Ser-473 and Thr-308 upon SCF treatment of osteoblasts, indicating a full Akt activation [17]. Activation of Akt by SCF was blocked by c-Kit inhibitor or silencing, indicating that SCF-activated Akt is downstream of c-Kit. More importantly, c-Kit inhibition almost abolished SCF-induced cyto-protective effects in osteoblasts. Based on these information, we propose that SCF activates c-Kit in osteoblasts, which mediates downstream Akt activation to protect osteoblasts from oxidative damages.

In summary, we have identified a new SCF-c-Kit-Akt physiologic pathway that protects osteoblasts from H_2O_2-induced damages, and might minimize the risk of osteonecrosis by oxidative stress.

Competing interests

All other authors state no conflicts of interest.

Acknowledgment

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References

[1] C.C. Chang, Y.S. Liao, Y.L. Lin, R.M. Chen, Nitric oxide protects osteoblasts from oxidative stress-induced apoptotic insults via a mitochondria-dependent mechanism, J. Orthop. Res. 24 (2006) 1917–1925.
[2] Y.H. Park, D.W. Han, H. Suh, G.H. Ryu, S.H. Hyon, B.K. Cho, J.C. Park, Protective effects of green tea polyphenol against reactive oxygen species-induced oxidative stress in cultured rat calvarial osteoblast, Cell Biol. Toxicol. 19 (2003) 325–337.
[3] Y. Zhu, J. Zhou, R. Ao, B. Yu, A-769662 protects osteoblasts from hydrogen dioxide-induced apoptosis through activating of AMP-activated protein kinase (AMPK), Int. J. Mol. Sci. 15 (2014) 11190–11203.
[4] C. She, L.Q. Zhu, Y.F. Zhen, X.D. Wang, Q.R. Dong, Activation of AMPK protects against hydrogen peroxide-induced osteoblast apoptosis through autophagy induction and NADPH maintenance. new implications for osteonecrosis treatment?, Cell Signal. 26 (2014) 1–8.
[5] B. Chabot, D.A. Stephenson, V.M. Chapman, P. Besmer, A. Bernstein, The proto-oncogene c-kit encoding a transmembrane tyrosine kinase receptor maps to the mouse W locus, Nature 335 (1988) 88–89.
[6] C.E. Edling, B. Hallberg, C-Kit–a hematopoietic cell essential receptor tyrosine kinase, Int. J. Biochem. Cell Biol. 39 (2007) 1995–1998.
[7] L. Ronnstrand, Signal transduction via the stem cell factor receptor/c-Kit, Cell. Mol. Life Sci. 61 (2004) 2535–2548.
[8] P. Blume-Jensen, R. Janknecht, T. Hunter, The kit receptor promotes cell survival via activation of PI 3-kinase and subsequent Akt-mediated phosphorylation of Bad on Ser136, Curr. Biol. 8 (1998) 779–782.
[9] H. Serve, N.S. Yee, G. Stella, L. Sepp-Lorenzino, J.C. Tan, P. Besmer, Differential roles of PI3-kinase and Kit tyrosine 821 in Kit receptor-mediated proliferation, survival and cell adhesion in mast cells, EMBO J. 14 (1995) 473–483.
[10] J.B. Fan, W. Liu, K. Yuan, X.H. Zhu, D.W. Xu, J.J. Chen, Z.M. Cui, EGFR trans-activation mediates pleiotrophin-induced activation of Akt and Erk in cultured osteoblasts, Biochem. Biophys. Res. Commun. 447 (2014) 425–430.
[11] A. Zecevic, H. Menard, V. Gurel, E. Hagan, R. DeCaro, A. Zhitkovich, WRN helicase promotes repair of DNA double-strand breaks caused by aberrant mismatch repair of chromium-DNA adducts, Cell Cycle 8 (2009) 2769–2778.
[12] B.H. Schrans-Stassen, H.J. van de Kant, D.G. de Rooij, A.M. van Pelt, Differential expression of c-kit in mouse undifferentiated and differentiating type A spermatogonia, Endocrinology 140 (1999) 5894–5900.
[13] E. Buchdunger, C.L. Cioffi, N. Law, D. Stover, S. Ohno-Jones, B.J. Druker, N.B. Lydon, Abl protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-kit and platelet-derived growth factor receptors, J. Pharmacol. Exp. Ther. 295 (2000) 139–145.
[14] M.M. Schittenhelm, S. Shiraga, A. Schroeder, A.S. Corbin, D. Griffith, F.Y. Lee, C. Bokemeyer, M.W. Deininger, B.J. Druker, M.C. Heinrich, Dasatinib (BMS-354825), a dual SRC/ABL kinase inhibitor, inhibits the kinase activity of wild-type, juxtamembrane, and activation loop mutant KIT isoforms associated with human malignancies, Cancer Res. 66 (2006) 473–481.
[15] G. Song, G. Ouyang, S. Bao, The activation of Akt/PKB signaling pathway and cell survival, J. Cell Mol. Med. 9 (2005) 59–71.
[16] C. Moller, J. Alfredsson, M. Engstrom, H. Wootz, Z. Xiang, J. Lennartsson, J.I. Jonsson, G. Nilsson, Stem cell factor promotes mast cell survival via inactivation of FOXO3a-mediated transcriptional induction and MEK-regulated phosphorylation of the proapoptotic protein Bim, Blood 106 (2005) 1330–1336.
[17] D.A. Altomare, J.R. Testa, Perturbations of the AKT signaling pathway in human cancer, Oncogene 24 (2005) 7455–7464.