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Foe turned friend: multiple functional roles attributable to hyper-activating stem cell factor receptor mutant in regeneration of the haematopoietic cell compartment

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Abstract

Objectives: Stem cell factor receptor, c-kit, is considered to be the master signalling molecule of haematopoietic stem cells. It develops the orchestral pattern of haematopoietic cell lineages, seen by its varying degree of omnipresence in progenitors, lineage committed and mature cells. We have investigated the effect of over-expressing c-kit on early recovery of the haematopoietic compartment, in irradiated hosts.

Materials and methods: Normal bone marrow cells (BMCs) were transfected with Kit_{wt} (wild-type c-kit) or its variant Kit_{mu} (asp814tyr) by electroporation. Lethally irradiated mice were transplanted with normal or transfected congeneic BMCs. The effect of ectopic expression of c-kit on haematopoietic cell recovery was determined by analysing donor-derived cells. Furthermore, effects of both types of c-kit over-expression on progenitor and lineage-committed cells were examined by flow cytometric analysis of Sca-1 and lineage-committed (Lin^+) cells respectively.

Results: Hyper-activating Kit_{mu} significantly improved recovery of the haematopoietic system in irradiated hosts. *In vivo* results showed that the donor-derived $c\text{-}kit^+$ cell population was increased to more than 3-fold in the case of Kit_{mu}-transfected cells compared to normal and Kit_{wt} over-expressing BMCs. In general, survival of progenitor and committed cell was improved in the Kit_{mu} over-expressing system compared to the other two cohorts.

Conclusion: These results suggest that recruitment of the hyper-activating variant of c-kit (Kit_{mu}) lead to early recovery of the bone marrow of lethally irradiated mice.

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Introduction

Haematopoietic stem cells (HSCs) are embodied with immense capability to construct a complete haematopoietic compartment from a single cell, which acts as the primitive powerhouse (1,2). Radiation-induced damage of the bone marrow (BM) leads to myelodysplasia, resulting from death of progenitors, with consequent loss of functional cells. The current approach to successful management of treating malignancy includes radiation and/or chemotherapy. This may result in depletion of stem cells, organ failure and late effects that might contribute to malignant transformation (3).

Patients undergoing radiation are given haematopoietic cell therapy from three major sources: (i) autologous/allogeneic BM, (ii) cytokine mobilized autologous peripheral blood, and recently (iii) cord blood. These sources of cells have several limitations such as improper HLA-matched donor cells, requirement of immunosuppressant treatment, recurrence of tumours and requirement of high dosage of cells for transplantation. The above limitations are clinically manifested as early effects (for example, mucositis, graft-versus-host disease, haemorrhagic cystitis, lung injury and veno-occlusive disease) and late effects (for example, musculoskeletal effects, ocular effects, endocrine effects and neurocognitive and neuropsychological effects), thus demanding faster recovery of the haematopoietic system.

Early recovery of the haematopoietic system from lethal irradiation is strictly limited due to G_0-silence or quiescence nature of HSCs. Hence, an alternative strategy is expected to be beneficial by engineering dormant haematopoietic cells for initiation of early signalling events. Although many HSC-specific transcription factors, growth factor receptors and corresponding ligands are known to activate haematopoietic cells, recruitment of a master signalling switch would ensure stage-specific regeneration of cells (4–6).

Previous studies have shown that viral vector-based gene delivery in HSCs has many drawerbacks (7–9). Primitive HSCs ($Lin^{-}Sca\text{-}1^{+}c\text{-}kit^{+}$; LSK), which are only 0.5% of total BM cells, have densely packed DNA inside intact nuclear membranes. This renders them difficult to transfect by a viral-mediated gene transfer method. The second obstruction is seen as post-maintenance of these modified populations *in vivo* for short-term as well as long-term gene expression, as a result of competition between transduced and non-transduced cells. This has been shown in the case of patients with chronic granulomatous conditions infused with gp91phox-transduced purified $CD34^{+}$ peripheral blood cells (7). The third and most severe problem associated with viral gene therapy is development of oncogenesis arising from insertional mutagenesis. Many gene therapy studies have shown development of leukaemia in patients who had undergone treatment for severe combined immunodeficiency (8,9).

Considering the above shortfalls of virus-mediated gene transfer, we propose a protocol of electroporation-mediated, transient over-expression, of a master signal transducer $c\text{-}kit_{wt}$ and its hyper-activating variant [Kit_{mu} (asp814tyr)] to reconstitute the haematopoietic system. The electroporation method is known to induce less cell death as cells do not experience chemical toxicity as in the case of chemical-mediated transfection (10–12). Earlier studies have confirmed $c\text{-}kit_{wt}$ as the key receptor for growth and survival of HSCs (13,14). Under normal circumstances, c-kit is activated by its ligand stem cell factor (SCF). In humans, constitutive activation of c-kit in neoplastic mastocytosis has been shown to bypass SCF interference in downstream signalling due to clustering of gain-of-function mutation Asp816 to Val in the Kit activation domain (15). Recently, we have predicted that certain activation loop residues in c-kit are crucial for interaction of kinases with Shp-1 for destabilization. Point mutation in stretches of Lys818 to Ser 821 and Thr847 to Glu849 in the Kit catalytic domain may lead to generation of a hyperactive variant of c-kit (16). In the mouse, Asp814 to Tyr mutation of the Kit activation loop results in over-expression of kinases as by degradation of tyrosine phosphatase (Shp-1) (17). Hyperactive Kit kinase has been reported to be associated with constitutive activation of phosphoinositide-3 kinase (PI3 kinase), signal transducer and activator of transcription 3 (STAT3), and nuclear factor kappa B (NF-κB) signalling pathways [16–18]. Cells affected were also independent of SCF for growth (18). These results suggest that recruitment of a hyper-activating mutant like Kit_{mu} (asp814tyr) would influence downstream signalling in lethally irradiated hosts to facilitate haematopoiesis.

Materials and methods

Animals

Six- to eight-week-old C57BL/6J [Ptprcb (Ly5.2)] and BL6.SJL [Ptprcc (Ly5.1)] mice were used in this study. Mice were obtained from the Jackson Laboratory (Bar Harbor, Maine, USA, http://www.jax.org) and maintained in our institute's experimental animal facility. During the experiments, mice were kept in an isolator and fed autoclaved acidified water and irradiated food *ad libitum*. All experiments using mice were conducted according to the procedures approved by the Institutional Animal Ethics Committee.

Isolation of BM cells

BMCs were isolated by flushing two tibia and two femurs from 6- to 8-week-old Ly5.1 mice, and erythrocytes were lysed by treatment with Gey's solution (19). Cell suspension was incubated in a tissue culture plate for 12 h to remove adhered stromal cells. Prior to electroporation, non-adherent viable cells were counted using the trypan blue dye exclusion method.

Electroporation of BM cells

BMCs were cultured for 12 h at 37 °C in a CO_2 incubator. Electroporation of cells (10×10^6 per cuvette) was performed using a Gene Pulser II Biorad electroporator (Hercules, CA, USA) at 1.7 kV/cm current and 400 μs pulse length. A custom-made minipulse chamber (with capacity of high cell density) having rectangular stainless steel electrodes (4 mm) was used for electroporation. Polyethylene glycol-purified pcDNA3.1-Kit constructs were diluted in electroporation buffer [10 mM HEPES, IMDM supplemented with 5% FCS and 5% FBS] at a concentration of 50–100 μg DNA/ml. To avoid post-pulse apoptosis, electroporated cells were washed and incubated at 37 °C in the 5% CO_2 incubator for 15 min. Incubation of post-pulsed cells is believed to facilitate resealing of pores (10,11).

Culture of Ly5.1 BMCs

Electroporated cells were cultured in Iscove's modified Dulbecco's medium (IMDM), supplemented with 10% FCS, 50 ng/ml murine SCF and 10 ng/ml interleukin-3 (IL-3) (PeproTech Asia, Rehovot, Israel, http://www.peprotech.com). Cells were cultured for different times at 37 °C in a 5% CO_2 incubator and viable cell number was determined.

Flow cytometry

Cells were labelled with antibodies and analysed according to methods described in our previous study (20). Antibodies used here were anti-: Sca-1/FITC, c-kit/phycoerythrin-Cy5 (BD Pharmingen, San Jose, CA, USA), CD45.1/PE-Cy5 and CD16/32 (eBiosciences, San Diego, CA, USA). Lineage antibody (CD5, CD45R, CD11b, Gr1, Ter119, 7-4) mixture was procured from Miltenyi Biotec (Gladbach, Germany). Streptavidin-PE and isotype control antibodies were also procured from BD Pharmingen. Prior to labelling with PE-conjugated antibodies, FcIIγR was blocked by incubating cells with CD16/32 antibodies for 15 min at 4 °C. Cells were analysed using customized BD-LSR apparatus (BD Biosciences, San Jose, CA, USA).

Haematopoietic reconstitution

Ten million Ly5.1 BMCs transfected with Kit_{wt} or Kit_{mu} (asp814tyr) were injected into mouse tail veins in two groups of animals (Ly5.2), previously irradiated at 900 cGy (1.96 cGy/s). The same number of normal (untransfected) BMCs was also transplanted in a separate group of irradiated mice, as controls. To evaluate marrow reconstitution, mice were killed at 48, 72, 96 and 144 h after transplantation. Donor-derived BM cells from each group of mice were analysed by flow cytometry for haematopoietic reconstitution and lineage commitment.

Cell metabolic activity

Cell metabolic activity was assessed by the tetrazolium 3-(4,5-dimethylthiazol-2-yl)-2,5 phenyltetrazolium bromide test (MTT) (21). Ten thousand cells (all types) were plated in triplicate wells of 96-well plates and cultured for 48 and 72 h. At each time interval, cultures were terminated for performing MTT assays (21). Colour intensity of formazone derivatives was measured spectrophotometrically at 540 nm, using a microplate reader. Greater colour intensity indicated higher metabolic activity and more viable cells. Viable cell number was also counted using the trypan blue dye exclusion method.

Statistical analysis

Results of multiple experiments were reported as mean ± SEM. One-way ANOVA and *post hoc* Tukey tests were performed for statistical analysis, with $P < 0.05$ being considered statistically significant.

Results

Efficiency of electroporation

Gene transduction efficiency into BMCs is normally very low, as the cells are highly heterogeneous. At 48 h of culture, Kit_{wt}-electroporated cells showed around 9% more c-kit expression compared to untransfected BM cell populations (Fig. 1a). Initially, the electroporation was conducted using different combinations of voltage and pulse length (data not shown). Combination of 1.7 kV/cm and 400 μs pulse length was found to be optimal for transfection. Post-electroporation cell death due to the effect of colloid causing osmotic swelling, was minimized by using high cell density. Prior to transfection, SCF was added to the culture medium for three major purposes: (i) to ensure exit of cells from a quiescent stage and to render them ready for DNA uptake, (ii) to provide a secondary anti-apoptotic signal for transduced cells, to protect the smallest fraction of the transfected population (22), and (iii) also to induce apoptosis of any terminally differentiated population to reduce unnecessary uptake of DNA by these cells.

Effect of c-kit ectopic expression on short-term cultures

Bone marrow contains a repertoire of c-kit^+ cells, but with significant distribution of different cell populations at various stages of haematopoiesis (23). We expected that ectopic expression of c-kit in murine BMCs should improve the number of primitive as well as committed progenitors. Previous results have shown that fresh BM contains 8–11% c-kit-expressing cells. Cultured BMCs expressed 7.57 ± 0.3% ($n = 3$) c-kit^+ cells, which increased to 11.7 ± 0.35% ($n = 3$) in c-kit-transfected BMCs (Fig. 1b,c). Interestingly, Kit_{mu} (asp814tyr)-transfected BMCs showed an approximately 2.5-fold increase in c-kit^+ cells in comparison to untransfected control cells (Fig. 1b,c). These results suggest that the hyper-activating nature of this mutant significantly ($P < 0.002$) enhanced the proportion of c-kit^+ cells compared to control BMCs (Fig. 1c).

Effect of c-kit over-expression on proliferation of donor-derived cells

Studies on rescue of haematopoietic stem cells from injury, in the recent past, have revealed that expression of

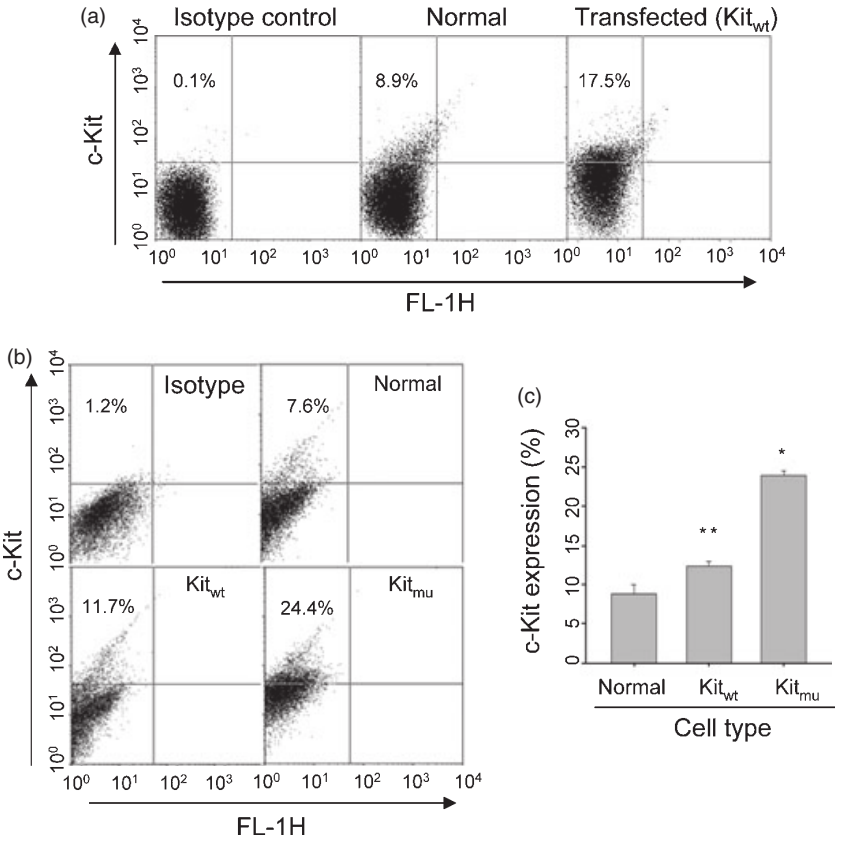

Figure 1. Electro-transfection of c-kit into bone marrow cells. (a) Efficiency of electroporation was evaluated in BMCs after 48 h transfection with Kit_{wt} plasmid. Expression of c-kit was analysed by flow cytometry. (b) Comparison of c-kit expression in normal and transfected (Kit_{wt} and Kit_{mu} plasmids) BMCs at 48 h of culture. Representative dot plots show that expression of c-kit receptor was more in the case of Kit_{mu}. (c) Bar diagram demonstrates statistical analysis of the results shown in (b). Number of experiment (n) = 3. $^{**}P < 0.05$; $^{*}P < 0.002$.

certain stem cell-specific genes, such as the *c-myc* proto-oncogene, can control balance between self-renewal and differentiation (24). It has also been hypothesized that ectopic expression of c-kit might be able to support extensive proliferation of donor BMCs in a lethally irradiated host. To show that, lethally irradiated Ly5.2 mice were transplanted with transiently transfected cells of Ly5.1 mouse. Representative dot plots for donor-derived cells at two different post-transfection times are shown in Fig. 2a. Chimaerisms of donor-derived cells were significantly increased in samples of transfected cells. At 72 h after transplantation, $Ly5.1^+$ cell population of wild-type cells was $2.8 \pm 0.4\%$ ($n = 4$), which increased to $7.3 \pm 0.4\%$ ($n = 4$) and $22.0 \pm 0.9\%$ ($n = 4$) in cases of Kit_{wt}- and Kit_{mu} (asp814try)-transfected donor cells respectively (Fig. 2b). The above values remained elevated even at 96 h after transplantation. Total $Ly5.1^+$ mononuclear cells sampled from pairs of tibias and femurs, after 72 h of transplantation with wild type, Kit_{wt}- and Kit_{mu}-transfected donor cells, were $(19.3 \pm 1.05) \times 10^6$, $(24.7 \pm 1.2) \times 10^6$ and $(30 \pm 1.16) \times 10^6$ respectively. The above results showed a significant ($P < 0.05$) proliferation advantage of donor cells over-expressing wild-type or mutant c-kit proto-oncogene, compared to normal cells. The results also reveal hyper-proliferative properties of BMCs transfected with Kit_{mu} (asp814try). Flow cytometry analysis not only showed the proliferative advantage of $Ly5.1^+$ cells, but also revealed that, in case of mutant c-kit over-expression, $c\text{-}kit^+$ cells were maintained at high level (Fig. 2c). Overall, these results suggest that c-kit over-expression leads to proliferation of grafted cells.

Effect of c-kit ectopic expression on self-renewal of progenitor cells

Earlier studies have suggested that a second signal from c-kit, which complements Jak2 is essential for triggering self-renewal of HSCs (25–27). In mouse, stem cell antigen-1 (Sca-1) is expressed on progenitor stem cells. Hence, we examined the effect of c-kit (wild type and mutant) over-expression on $Sca\text{-}1^+$ cells in culture. Representative dot plots show that in the culture of normal BMCs, Sca-1 expression was marginally increased in the presence of SCF (Fig. 3a). There was some increase in Sca-1-expression in Kit_{wt}-transfected cells in the presence of SCF as compared to its absence, which may not be statistically significant (Fig. 3b). $Sca\text{-}1^+$ cells were significantly ($P < 0.001$) increased in cases of Kit_{mu} transfection. At 48 h culture, $Sca\text{-}1^+$ cells increased from $9.0 \pm 0.45\%$ to $25 \pm 0.8\%$ (Fig. 3b). Proliferation of

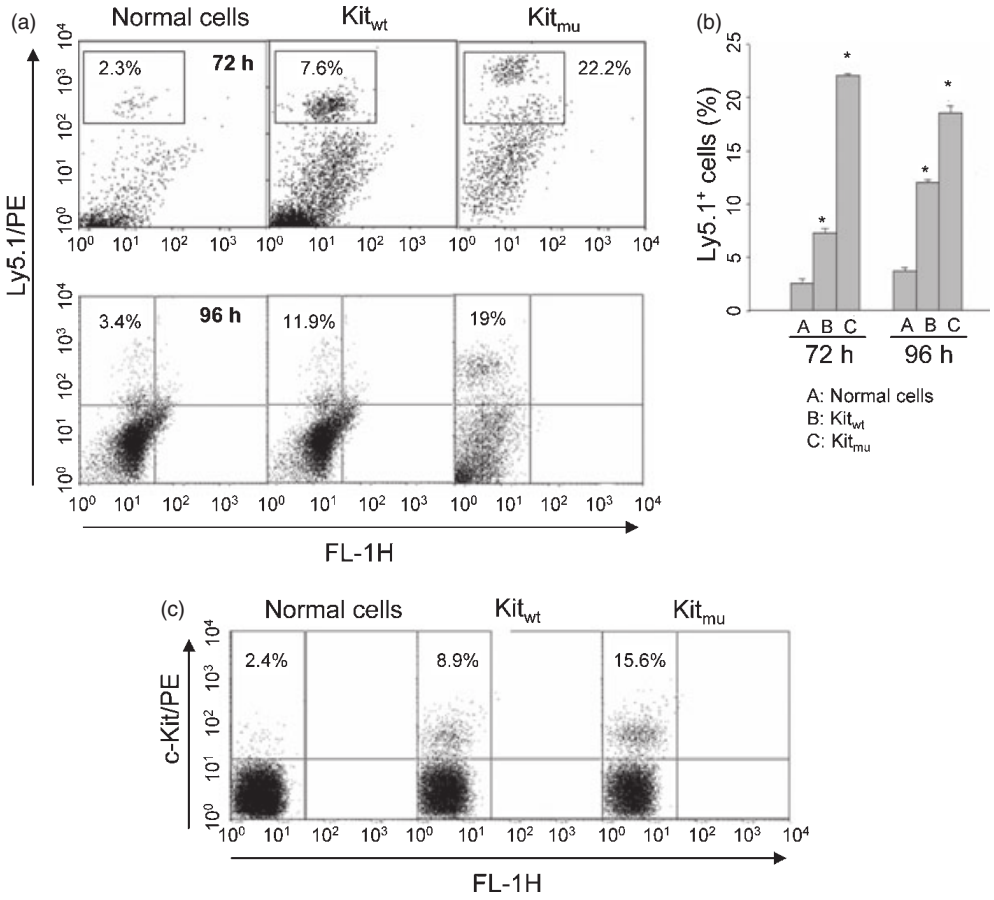

Figure 2. Effect of c-kit ectopic expression on engraftment/proliferation of donor cells. Ten million cells (per mouse) were transplanted into three groups of lethally irradiated animals. (a) Flow cytometry analysis of donor-derived cells (Ly5.1$^+$) at 72 and 96 h of transplantation. Representative dot plots compare donor cells in lethally irradiated recipients transplanted with normal, Kit$_{wt}$ and Kit$_{mu}$ over-expressing cells. (b) Bar diagram shows statistical analysis of results depicted in (a). Significant increase in Ly5.1$^+$ cells was observed in cases of c-kit (wild and mutant) over-expressing donor cells compared to normal BMCs. (c) Flow cytometry analysis of c-kit expression. Representative dot plots show expression of the receptor at 96 h of transplantation. More c-kit$^+$ cells were observed in case of Kit$_{mu}$-transplanted cells than in the other two sample types. All experiments were conducted twice using three mice in each group. *$P < 0.005$.

Kit$_{wt}$- and Kit$_{mu}$-transfected cells was compared to wild-type (untransfected) cells. It was revealed that transfection with Kit$_{mu}$ plasmid lead to proliferation of BMCs in SCF-supplemented culture (Table 1). These analyses suggest that Kit$_{mu}$ (asp814try) transfection increased the self-renewing capacity of the progenitor stem cells, compare to that obtained in the other two cases.

Effect of c-kit over-expression on lineage commitment

One of the aims of c-kit over-expression was to facilitate early haematopoietic differentiation of donor cells in lethally irradiated hosts. To determine lineage commitment by c-kit ectopic expression, we cultured transfected cells for 72 h in the presence of SCF. The representative dot plots show that in the region of 37% wild-type cells were Lin$^+$, which increased to 67.5% and 80.5% for Kit$_{wt}$- and Kit$_{mu}$-transfected cells, respectively (Fig. 4a). These results probably suggest that Kit$_{wt}$ and Kit$_{mu}$ transfection maintained more differentiated phenotypes.

We also compared the lineage commitment of c-kit over-expressing cells in the transplantation model. In these studies, BM cells were analysed at 72, 96 and 144 h after transplantation with normal, Kit$_{wt}$- and Kit$_{mu}$-transfected cells. These results suggest that in Kit$_{wt}$- and Kit$_{mu}$-transplanted mice, lineage-committed cells were significantly increased compared to normal BMCs (Fig. 4b).

Effect of c-kit over expression on survival of cells

The BM is composed of haematopoietic progenitors, lineage-committed and terminally differentiated cells.

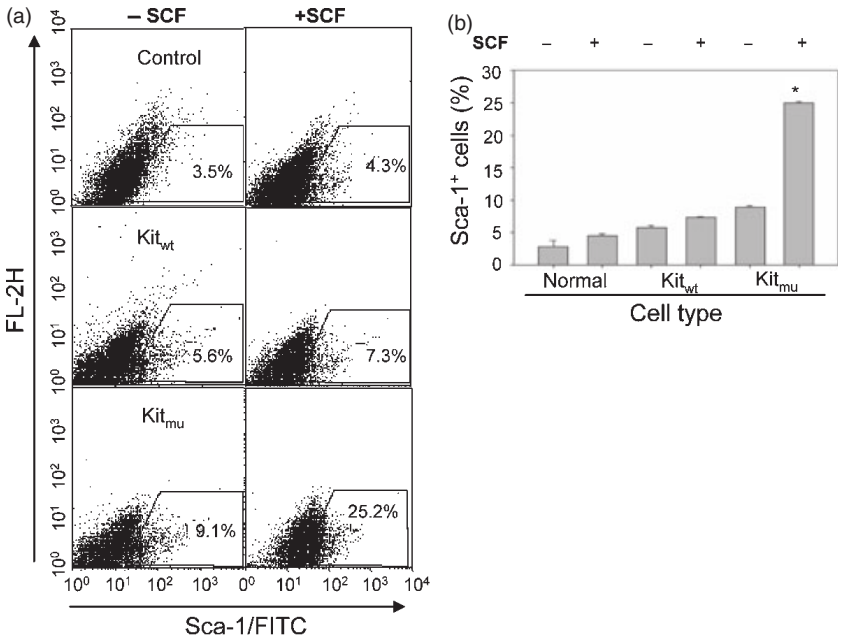

Figure 3. Self-renewal of progenitor stem cells (Sca-1$^+$) in normal and c-kit over-expressing cells in culture. Ten million cells of each kind were cultured for 48 h in the absence or presence of stem cell factor (SCF). (a) Flow cytometry analysis of progenitor stem cells. Representative dot plots show more Sca-1$^+$ cells in the c-kit over-expressing culture. Self-renewal of progenitor stem cells was considerably increased in Kit$_{mu}$ over-expressing cells in the presence of SCF. (b) Bar diagram shows statistical values of results in (a). Ectopic expression of Kit$_{mu}$ in BMCs significantly increased self-renewal of progenitor cells. $n = 3$. *$P < 0.001$.

Table 1. Number of mononuclear cells after 96 h of culture in the absence and presence of SCF

	Normal cells ($\times 10^6$ per culture)		Kit$_{wt}$-transfected cells ($\times 10^6$ per culture)		Kit$_{mu}$-transfected cells ($\times 10^6$ per culture)	
Starting cells ($\times 10^6$ per culture)	−SCF	+SCF	−SCF	+SCF	−SCF	+SCF
10.0	3.6 ± 0.3	4.3 ± 0.5	5.4 ± 0.63	7.6 ± 0.56	9.2 ± 0..85	23.9 ± 1.9

Lineage-committed cells uptake DNA during their cell cycle and they also undergo apoptosis (28,29). Earlier studies have shown that in HSCs, c-kit mediates complementary anti-apoptotic signals along with BCl-2 expression (22). Therefore, we assumed that forced expression of c-kit should influence survival efficiency of the cells. To evaluate effects of ectopic expression of c-kit directly on cell survival, untransfected and transfected BMCs were cultured for 72 h in the presence of SCF. The relative cell number was determined by MTT assay and viability by the trypan blue dye exclusion technique. Results show that metabolic activity and viability of Kit$_{mu}$ (asp814tyr) plasmid-transfected cells were significantly improved compared to control and even Kit$_{wt}$ over-expressing cells (Fig. 5a,b). These results suggest that the hyperactive mutant of c-kit improved cell survival and proliferation rates than the other two types.

Discussion

Low recovery of BM cells in irradiated hosts justifies immediate attention to achieve certain maneuvers in modulation of the haematopoietic system: (i) exit of HSCs from the quiescent state, (ii) proliferation to maintain the stem cell pool, (iii) onset of HSC differentiation into oligopotent progenitors, and finally (iv) formation of mature cells. Previous studies have described the crucial roles of c-kit in regeneration of different haematopoietic compartments by activating stage-specific signals for proliferation, differentiation and anti-apoptosis (13,14,22,23,27,30). The gain-of-function mutation in the c-kit kinase domain renders hyper-proliferative ability to its kinase activity, which may lead to haematological malignancies, whereas a loss-of-function mutation of c-kit can lead to developmental defects (31). Earlier studies have described that c-kit activation triggers important signalling switches in the haematopoietic hierarchy, and these are the PI3K dependent pathway in proliferation and differentiation, the JAK-2 and STAT3/5A-dependent pathway, and employment of FOXO3A by p85^{PI3K}-dependent pathway in self-renewal, and providence of secondary complementary anti-apoptotic signals for survival (27,30,32,33). On acquiring a constitutive point mutation, c-kit transduces altered signals, leading to constitutive activation of PI3K,

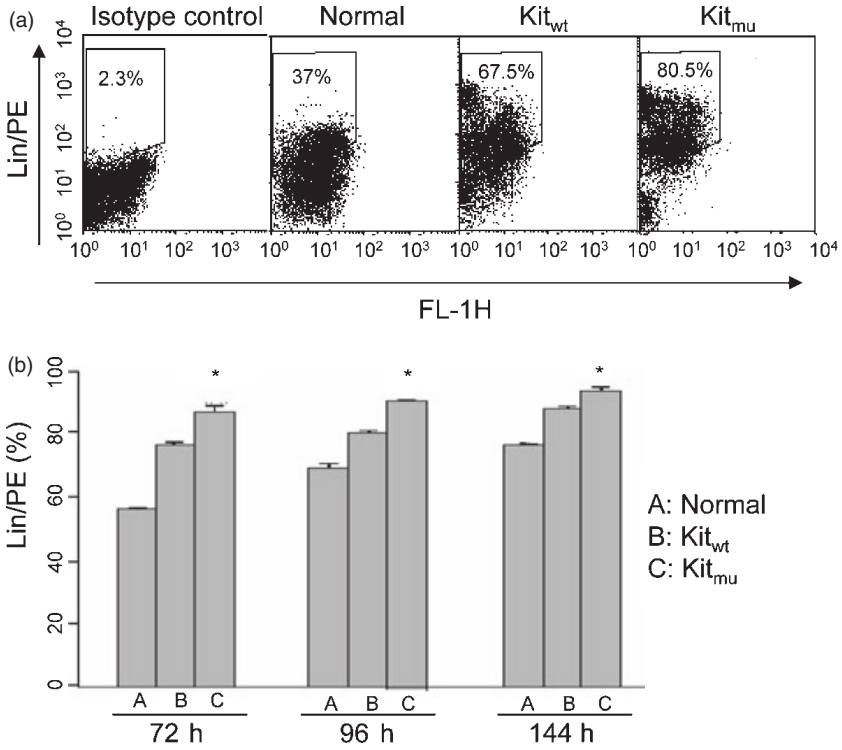

Figure 4. Effect of c-kit ectopic expression on lineage commitment. (a) Flow cytometry analysis of lineage commitment. Three million cells of each kind were cultured for 72 h in the absence or presence of SCF. Representative dot plots show more Lin$^+$ cells in cases of Kit$_{wt}$ and Kit$_{mu}$ over-expressing cells than normal BMCs (n = 3). (b) Ten million cells of each type were transplanted in three groups of lethally irradiated mice. Bar diagram shows comparative statistical values of flow cytometry analysis of lineage committed cells at three different times of transplantation. Results show a significant increase in Lin$^+$ cells in mice receiving Kit$_{mu}$-transfected cells compared to the other two categories. The number of mice in each group for each time point was three. *P < 0.005.

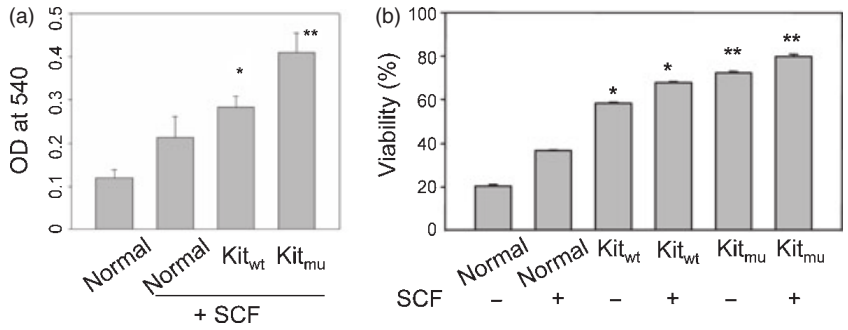

Figure 5. Effect of c-kit over-expression on cell proliferation and viability. (a) Ten thousand normal and transfected bone marrow cells (BMCs) were cultured in each well for 72 h in the presence of stem cell factor (SCF). Metabolic activity of cells was determined by MTT assay. Metabolic activity was significantly increased in c-kit over-expressing cells compared to normal BMCs. (b) Ten thousand normal and transfected BMCs were cultured in each well for 72 h in the absence and presence of SCF. Cell viability was determined by the trypan blue dye exclusion method. Viability of cells was significantly increased in c-kit over-expressing cells compared to normal BMCs. n = 3. *P < 0.01; **P < 0.005.

STAT3 and NF-κB pathways as part of the downstream events of HSC signalling (15,18,33).

To ensure early restoration of haematopoietic compartments in an irradiated host (by switching on stage-specific signals), we thought of targeting a multidimensional signal transducer, c-kit, and its hyper-activating mutant asp814tyr, in donor BMCs prior to transplantation. Accordingly, we performed transient over-expression of Kit$_{wt}$ and Kit$_{mu}$ (asp814try) in murine BMCs for enhancement of c-kit-dependent signalling. We followed electroporation method to transfer target genes, as this method seems to be simpler and more efficient than viral-based gene delivery systems (10,34). Furthermore, integration of a transgene into the genome is not the issue in the case of electroporation (35,36).

The present study describes utilization of multifunctional attributes of Kit$_{wt}$ and Kit$_{mu}$ for early regeneration of the haematopoietic system in lethally irradiated mice. Ly5.1$^+$ donor BMCs were genetically modified for transient over-expression of Kit$_{wt}$ and Kit$_{mu}$. We adopted

a transient transfection system, as an initial period of marrow recovery, is most crucial in cases of lethal irradiation. We expected that these transfected cells would reconstitute the bone marrow much more rapidly than normal BMCs. Besides, oncogenic transformation would not occur in this protocol as could be found in cases of viral-based gene delivery systems. Results suggested that mice transplanted with wild-type c-kit over-expressing BMCs recovered much faster than control cells, in terms of bone marrow cellularity. Again, the hyper-activating c-kit mutant showed better results than wild-type c-kit. Our study was limited to 6 days after post-transplantation as transiently transfected cells die or expel the foreign gene within that period, in the absence of selection pressure. Moreover, blood cytopenias are commonly occurring symptoms during the initial period of radiation.

Ectopic expression of Kit_{wt} and Kit_{mu} showed drastic changes in irradiated hosts at different stages of haematopoiesis compared to normal BMCs. The number of progenitor stem cells ($Sca\text{-}1^{+}$) was enriched, and simultaneously, lineage-committed cells (Lin^{+}) also increased. This was possible because of over-expression of c-kit, which has been implicated for stage-specific development of haematopoietic cells (13,14). The results also confirmed that in the case of Kit_{mu}, cell proliferation and differentiation were improved compared to Kit_{wt} cells. In culture experiments, we found interesting results when SCF was incorporated in the medium. The purpose of using SCF was to counteract apoptotic pathways activated due to transfection and mimicking an *in vivo* situation where SCF is available in the bone marrow microenvironment. Kit_{mu}-transfected BMCs also showed maximum cell survival efficiency and metabolic activity compared to the other two kinds of graft used in this study. Our *in vitro* and *in vivo* results were comparable. Our preliminary study demonstrates that over-expression of Kit_{mu} in donor cells can efficiently restore haematopoietic compartments in irradiated hosts. In other words, transient over-expression of the hyper-activating variant of c-kit drastically modulated haematopoiesis in lethally irradiated hosts. Thus, designing a hyper-activating mutant of c-kit could generate new therapeutic intervention of BM reconstitution in case of myeloablation.

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