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Interferon Regulatory Factor-2 Regulates Hematopoietic Stem Cells in Mouse Bone Marrow

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1. Introduction

Hematopoiesis is regulated by intrinsic gene-regulatory networks, ensuring the rapid production of differentiated blood cells for the immediate needs of embryos and generation of definitive hematopoietic stem cells (HSCs) that are required for life-long hematopoiesis. Homeostasis in bone marrow is dependent on the ability of HSCs to faithfully self-renew and to generate progenitor cells that undergo limited proliferation and give rise to terminally differentiated cells in the peripheral blood. HSCs are specialized to give rise to all elements of the blood system throughout life (Orkin & Zon, 2002, 2008a, 2008b) and are capable of self-renewal and differentiation into various lineages of the hematopoietic system to form all types of blood cells. Self-renewal is a tightly controlled process through which stem cells divide and generate daughter stem cells with properties identical to those of the mother cells. However, under certain conditions, HSCs differentiate into progenitor cells with less ability to self-renew. Since the discovery of stem cells, intense research aimed at understanding the genetic and molecular bases of self-renewal has identified candidate regulatory factors involved in the process of HSC self-renewal. These include cell-intrinsic regulators, such as transcription factors, signal transducers, cell-cycle inhibitors and surface receptors and cell-extrinsic regulators, such as the bone marrow niche and cytokines (He et al., 2009).

Interferon (IFN) is produced by cells of the immune system in response to challenges by agents, such as viruses, bacteria and tumor cells. IFNs suppress viral replication, have immunomodulatory activities and are used clinically to treat viral diseases and malignancies, such as chronic myeloid leukemia (CML)(Stark, 1998). Type I IFNs are induced by the genomes of many RNA viruses, and this induction can be mimicked by the double-stranded RNA mimetic polyinosinic-polycytidylic acid (poly [I:C]) (Darnell et al.

1994, Pichlmair et al. 2007). However, under steady-state conditions in the absence of infection, small amount of IFN are produced constitutively (Taniguchi & Takaoka, 2001). Recently, Essers et al demonstrated that chronic activation of IFN-α pathway impairs function of HSCs and acute IFN-α treatment promotes the proliferation of dormant HSCs in vivo, and the possibility for new application of type I IFN to target cancer stem cells is expected (Essers et al. 2009) .

Interferon regulatory factors (IRFs) constitute a family of transcription factors involved in regulating the development and functions of the immune system (Honda et al., 2006; Taniguchi et al., 2001). Interferon regulatory factor-2 (IRF-2) is a transcriptional repressor in the interferon system and is thought to function by competing with IRF-1. While IRF-2 acts as a repressor for interferon production, IRF-2 exists ubiquitously and is a positive regulator for H4, vascular adhesion molecule-1 (VCAM-1), CIITA, gp91 phox, Fas ligand, TPO receptor (Vaughan et al.1995, Jesse et al. 1998 , Xi et al. 1999, Luo & Skalnik 1996, Chow et al. 2000, Stellacci et al. 2004, Masumi et al, 2001). Previously, we demonstrated that IRF-2 expression into mouse bone marrow hematopoietic stem/progenitor cells induced megakaryopoiesis through CD41 promoter activation in an inflammatory states (Masumi et al. 2009). IRF-2 regulates cell growth and differentiation through the target gene promoters.

There are several studies of hematopoietic approaches using IRF-2-/- mice. The physiological role in lymphoid and hematopoietic development has been investigated in IRF-2-/-mice, in which a general bone marrow suppression of hematopoiesis and B lymphopoiesis has been reported (Matsuyama et al. 1993). Recently, a marked reduction of hematopoietic stem cells in IRF-2-/- mice involving a type I interferon-dependent mechanism was reported (Sato et al. 2009). The population of bone marrow Lin-c-Kit+Sca-1+ (KSL) cells is increased in IRF-2-/- mice because of the general enhancement of Sca-1-positive cells.

Herein, we show that an enhanced population of Sca-1-positive cells and reduced HSC activity in the Lin-Sca-1+c-kit+ fraction were detected in IRF-2-/-mouse bone marrow cells. HSC abnormalities in IRF-2-/- mice have been demonstrated to be due to elevated type I IFN signaling (Sato et al. 2009). IFN signaling enhances the Sca-1 expression and cell cycle progression of HSCs. It was shown that chronic IFN signaling enhances cell cycle progression of HSCs in IRF-2-/-mice, resulting in the loss of quiescent HSCs. However, our results reveal unknown HSC markers in bone marrow from IRF-2-/- mice. Our present findings demonstrate that IRF-2 acts on long-term (LT)-HSCs, not only through protective type I IFN responses, but also by directly regulating HSC cell-surface molecules.

2. Hematopoietic stem cells in bone marrow derived from interferon regulatory factor-2-deficient mice

To analyze the expression of IRFs in mouse bone marrow cells, Gr1/Mac1-positive, B220-positive, Ter119-positive/lineage (Lin)-negative and KSL (c-kit+Sca-1+Lin-) cells were isolated from mouse bone marrow cells by flow cytometry. Real-time polymerase chain reaction (PCR) analysis and in situ hybridization showed that IRF-2 was present in especially high levels in the CD34-KSL fraction compared with fractions from other lineages (Masumi et al., 2009). The CD34-KSL cells from mouse bone marrow were stained with anti-IRF-2 antibody and DAPI (Fig. 1).

IRF-2   DAPI   Merge

Fig. 1. CD34-KSL cells were stained with anti-IRF-2 as the primary antibody and then with anti-rabbit Alexa594 as the secondary antibody. Concurrently, cells were stained with DAPI.

2.1 Isolation and characterization of KSL cells

We showed that IRF-2 was highly localized in the mouse HSCs (CD34-Lin-c-kit+Sca-1+) in Fig. 1. To examine the role of IRF-2 in mouse hematopoietic stem cells, we isolated Lin-c-kit+sca-1+ (KSL) cells from the bone marrow of IRF-2-/-mice (Fig. 2A). The IRF-2-/- mice had a larger population of KSL cells than did wild-type mice because of enhanced expression Sca-1, which is downstream of interferon-α-receptor (IFNAR)-STAT1 signaling. Enhanced type I IFN signaling in IRF-2-/- mice induces Sca-1. Sca-1 cell-surface glycoprotein is used routinely as a marker of adult HSCs, allowing a >100-fold enrichment of these rare cells from the bone marrow of adult mice. The Sca-1 protein is encoded by the Ly-6A/E gene. This protein is highly inducible by IFNs -α, –β, and -γ, tumor necrosis factor (TNF) and interleukin-1 (IL-1) (Khan et al., 1990, 1993). The presence of a consensus sequence for IFN-γ-responsive elements has been reported to be localized to the Ly-6A/E genes promoter (Ma et al., 2001). Ito et al investigated that competitive repopulation assay using HSC from Sca-1-deficient mice and colony formation assay for Sca-1-deficient bone marrow. They demonstrated that Sca-1 is required for regulating HSC self-renewal and development of committed progenitor cells, megakaryocytes, and platelets (Ito et al. 2003). Bradfute et al investigated the effect of Sca-1 on HSC function and demonstrated that Sca-1 affects c-kit expression, the lineage fate of peripheral blood cells after transplantation, and may be dispensable for HSC self-renewal (Bradfute et al., 2005).We also observed higher populations of KSL cells in the spleens of IRF-2-/- mice (data not shown).

HSCs are contained within the CD150+CD48- population of KSL cells. There were far fewer CD150+CD48- cells in the KSL fraction in IRF-2-/- mice than in wild-type mice (0.97% versus 0.001%). In contrast, within the CD150+ CD48-Lin- subset, the fraction of KSL cells in wild-type mice and IRF-2-/- mice was 31.58% and 0.001%, respectively. Thus, the population of CD150+CD48-KSL cells was very low in IRF-2-/- mice (Fig. 2B). IRF-2 is known to be a transcription factor that attenuates type I IFN (IFN-α/IFN-β) signaling as indicated by the up-regulation of IFN-inducible genes in IRF-2-/-mice. IFN-α/IFN-β produced by plasmacytoid dendritic cells (DCs) in IRF-2-/- mice may have stimulated HSC proliferation, which resulted in loss of stem cells (Fig. 2C).

The number of KSL side-population (SP) cells was much lower in bone marrow cells from IRF-2-/- mice compared to those from wild-type mice (Fig. 3). We observed an increased cell number in the KSL fraction and a great reduction in the number of HSCs in the KSL fraction among the bone marrow cells of IRF-2-/- mice.
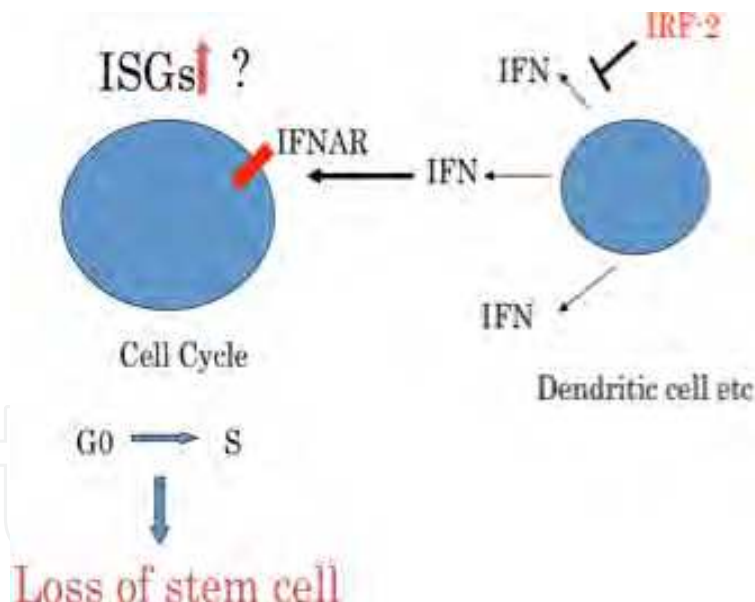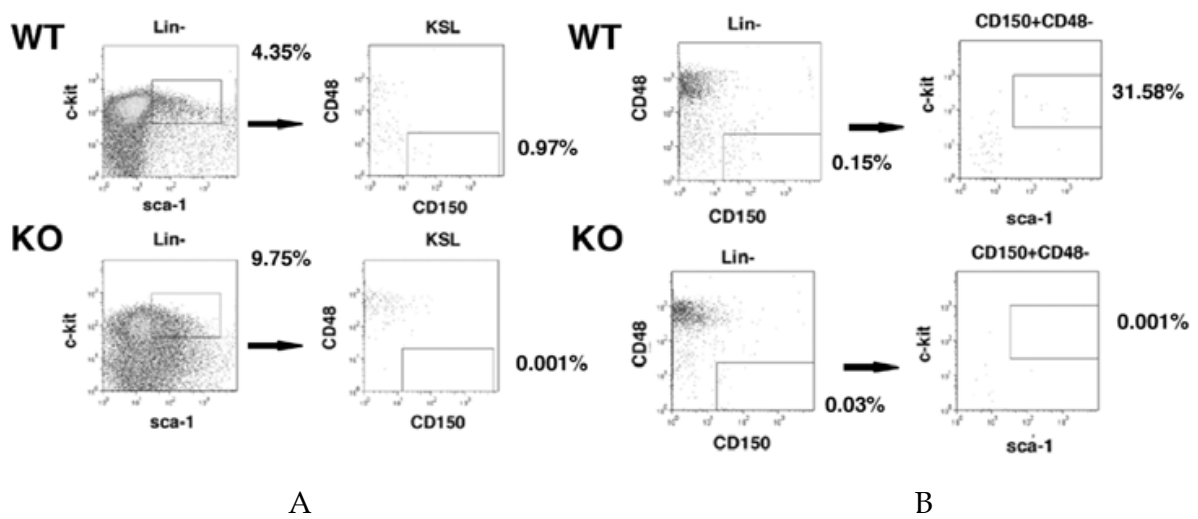
Fig. 2. (A) Lin-c-kit+sca-1+ cells were isolated from wild-type (WT) and IRF-2-/- bone marrow cells (KO), and then Lin-CD48-CD150+ cells were isolated from the KSL fraction. (B) Lin-c-kit+sca-1+ cells were isolated from Lin-CD48-CD150+ cells derived from the bone marrow cells of IRF-2-/- mice. (C) Chronic IFN stress model in KSL cells of IRF-2-/- mice. ISGs : Interferon-stimulated genes

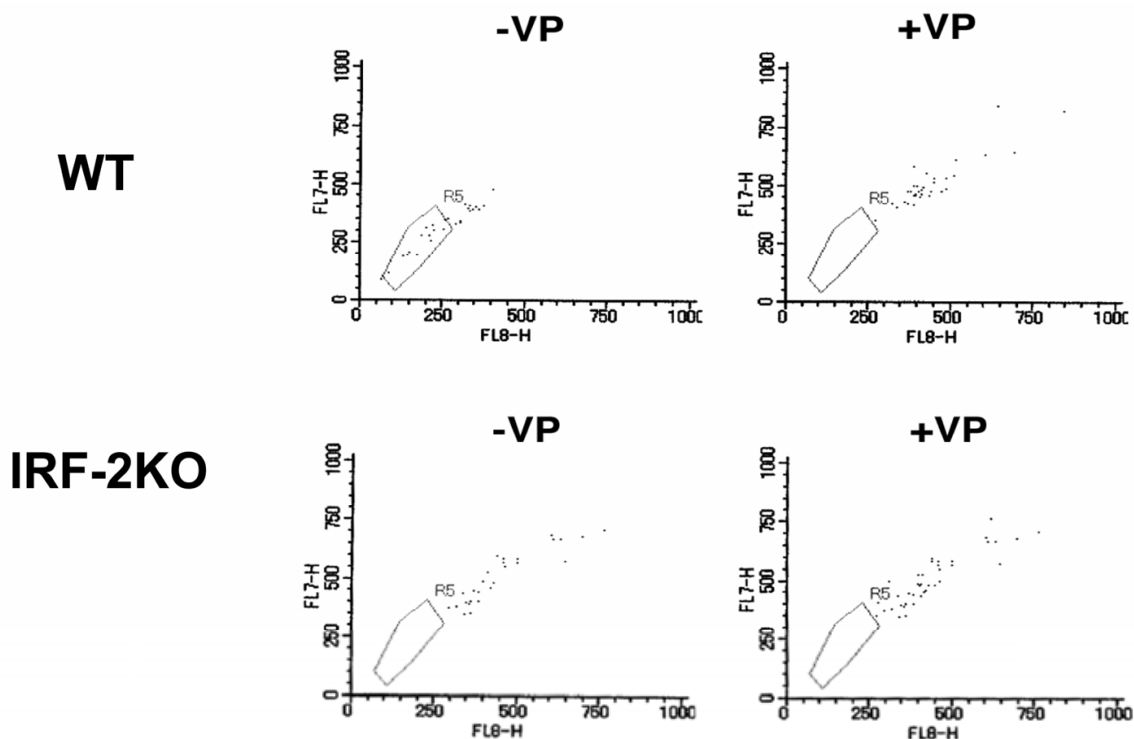Fig. 3. Reduction in side population of KSL cells in the bone marrow cells from wild-type (WT) and IRF-2-/- mice (IRF-2KO). VP (verapamil) treatment eliminated the side population.

2.1.1 In vitro differentiation of KSL

To analyze the population of the KSL fraction in bone marrow from IRF-2-/- mice, an in vitro colony-forming assay was performed. The assay showed enhanced granulocyte/macrophage progenitor activity and reduced numbers of megakaryocyte progenitors in KSL derived from bone marrow of IRF-2-/- mice (Fig. 4). We did not see any difference for erythrocyte progenitors in the bone marrow derived-KSL cells in either wild-type or IRF-2-/-mice.

To compare ex vivo expansion of HSC between wild type and IRF-2-/-mice, KSL cells were plated at a density of 1 cell/well in Terasaki plates in 20 µL serum-free medium. The Terasaki single colony assay showed the significant colony formation activity in KSL cells from IRF-2-/- mice although its activity is less than that of wild-type mice (Fig. 5). In vitro culture assay, KSL cells from IRF-2-/- mice make colonies in the presence of cytokines, despite of near complete reduction of HSC population (Fig.2).
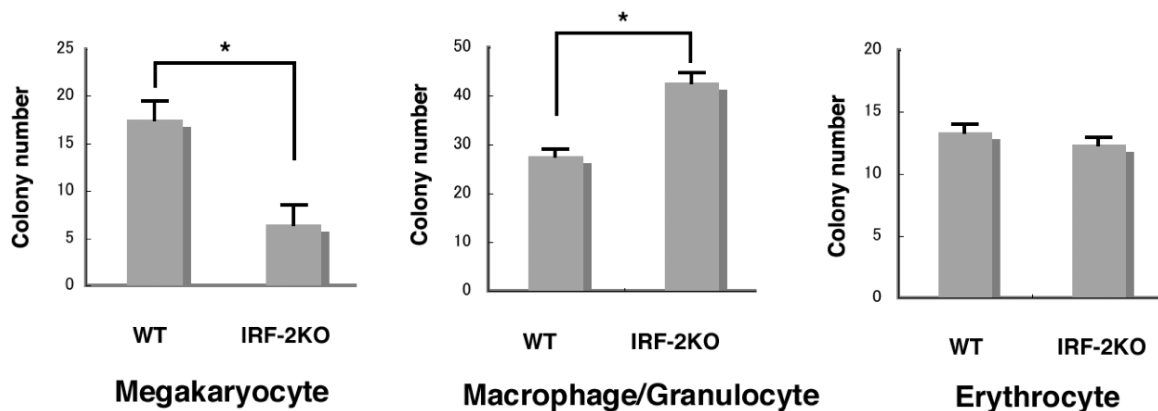
Fig. 4. Clonogenic progenitor assay. Two hundred KSL cells were subjected to a colony-forming unit-granulocyte/macrophage CFU-GM assay in methylcellulose medium M3231 (Stem Cell Technologies, Vancouver, BC, Canada) consisting of 1% methylcellulose, 30% fetal calf serum (FCS), 1% bovine serum albumin (BSA), 10 ng/mL stem cell factor (SCF), 25 ng/mL Flt ligand, 25 ng/mL thrombopoietin (TPO), 5 ng/mL IL-3 and 25 ng/mL granulocyte colony-stimulating factor (G-CSF). For the burst-forming units-erythroid (BFU-E) assay, 200 KSL cells were cultured in M3231 consisting of 1% methylcellulose, 30% FCS, 1% BSA, 50 ng/mL SCF, 50 ng/mL TPO and 5U/mL erythropoietin (EPO) for 7 days. To perform the mouse CFU-megakaryocytic (Mk) assays, 4×10^3 KSL cells were mixed with Megacult-C 04900 together with 1.1 mg/mL collagen, 50 ng/mL TPO and 10 ng/mL IL-3 in 0.75 mL and added to the wells of chamber slides (177429, LAB-TEK Brand Products). Cells were cultured at 37°C in an incubator with an atmosphere of 5% CO_2 and >95% humidity for 7 days. The chamber slides were placed in acetone solution to fix the cells and dried, and the dried slides were then stained with acetylthiocholiniodide solution (Sigma, St Louis, MO). After they were stained with hematoxylin, megakaryocyte colonies were counted. *$P<0.05$ (Student *t* test). Data are representative of two independent experiments (mean ±SD).
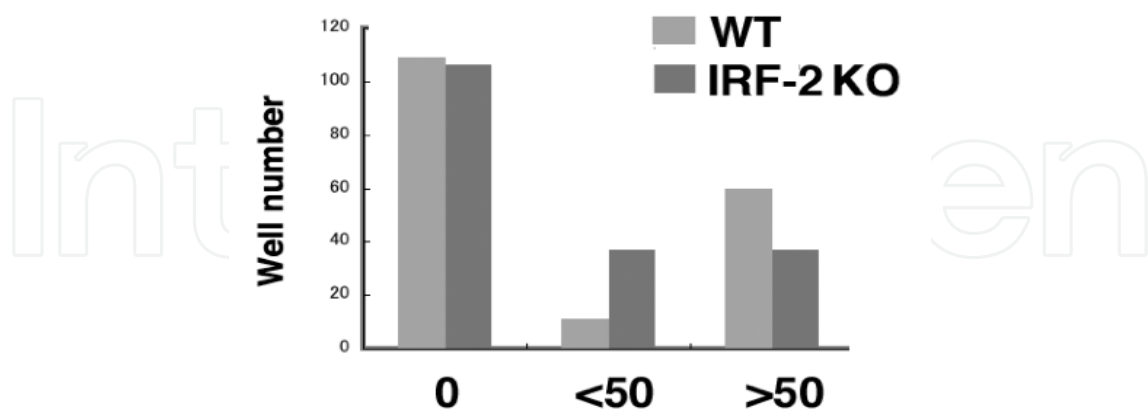

Fig. 5. Terasaki single colony assay. The KSL fraction from wild-type and IRF-2-/- bone marrow cells was fractionated to single cells in Terasaki plates by cell sorter (JSAN). Single cells were cultured with cytokines (hTPO, mSCF, mIL-3 and mFlt-3 ligand) containing 10% BSA and 2-mercaptethanol (0.01 M) in X-VIVO medium for 10 days. Colonies in each well were analyzed. <50 indicates wells that contain under 50 colonies in one well. >50 indicates wells that contain over 50 colonies. 0 indicates the wells containing no colony.

HSCs in wild-type mice were predominantly in a quiescent, intracellular Ki67-negative (icKi67 Hoechst low) G_0 phase. Quiescent cells were observed more frequently in KSL cells derived from IRF-2-/- mice, although the population of HSCs was much smaller than that of wild-type mice (Fig. 6).

2.1.2 Gene expression of KSL cells

Next, we investigated the KSL-specific gene expression in KSL cells derived from bone marrow cells of IRF-2-/-mice. Expression levels of GATA-2 and Tie2 in IRF-2-/- mice were similar to those of wild-type, but p57 expression was much lower than that of wild-type (Fig. 7A). Reduced p57 gene expression is associated with decreased numbers of HSCs in the KSL population, and the increased number of cells in G_0 phase may be associated with an increased frequency of quiescent KSL cells in bone marrow cells from IRF-2-/- mice (Fig. 6). When types I and II IFN were analyzed, expression of IFN-γ, but not of IFN-α and -β was decreased in the KSL fraction of IRF-2-/- mice under no stimulation (Fig. 7BC). Expression of Sca-1 was enhanced in the KSL fraction and in whole bone marrow in the IRF-2-/- mice. We examined PKR, TNF-a, adenosine deaminase 1 (ADAR1) expression which is known to be a suppressor of interferon signaling (Hartner et al. 2009) and Bmi1, which is down regulated in IRF-2 deficient HSC (Sato et al 2009). Expressions of ADAR1, PKR, Bmi1 and TNF-α were comparable between wild-type and IRF-2-deficient mice (Fig. 7C).
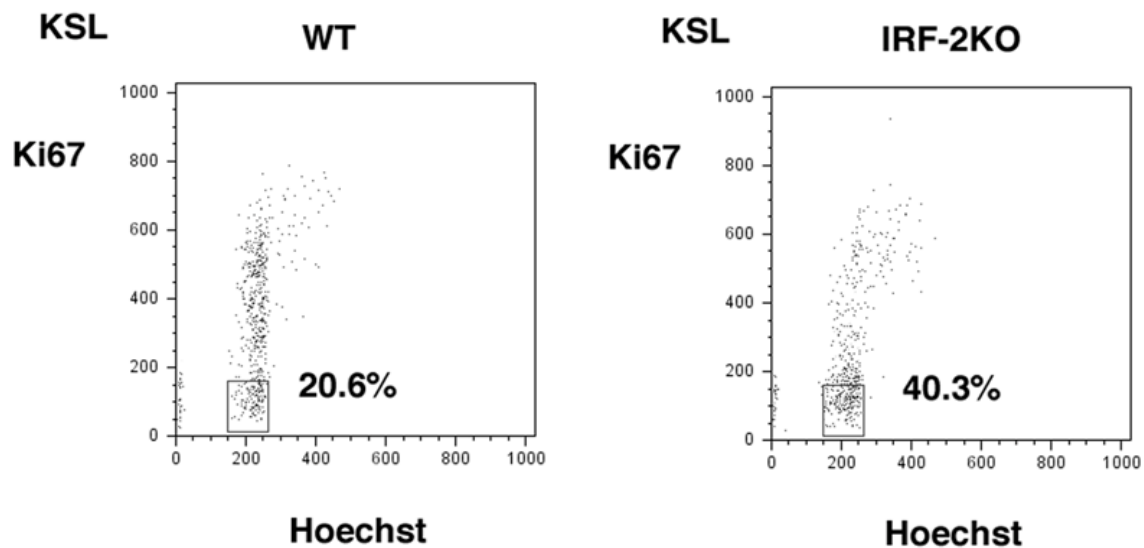

Fig. 6. Cell cycle analysis. Populations of KSL cells were isolated from bone marrow cells of IRF-2-/-mice and stained with Ki67 and Hoechst. Numbers indicate the percentage of cells in G_0 phase. Data are representative of two independent experiments.
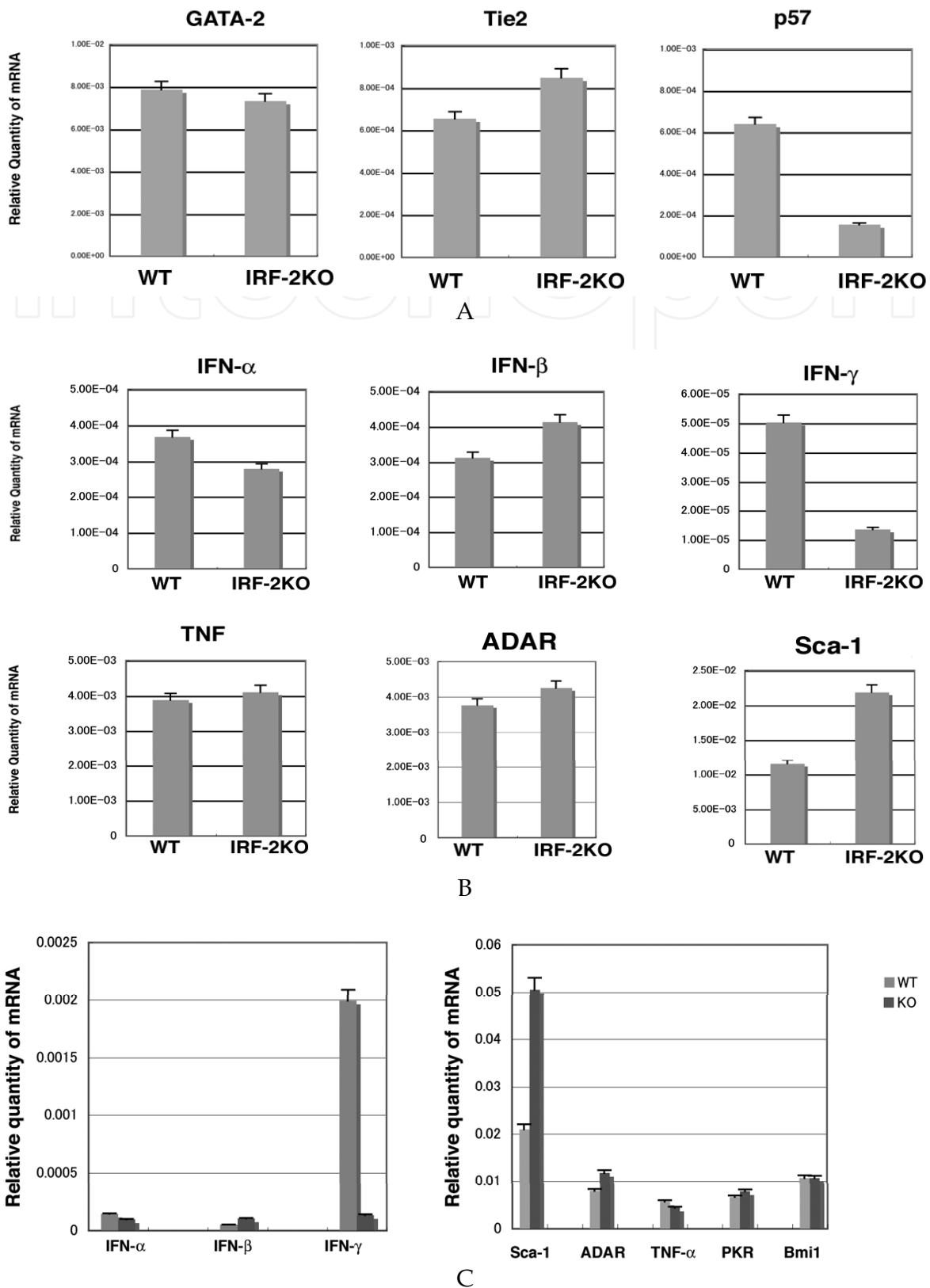
Fig. 7. Gene expression in KSL (A and B) and bone marrow cells (C) from wild-type and IRF-2-/- mice. Data represent the mean ± SD of triplicate reactions and are representative of two independent experiments.

2.1.3 Transplantation

To examine the functional properties of IRF-2-/- KSL cells, transplantation analysis was performed. In competitive repopulation assays, a constant number (1x10^5) of wild-type competitor cells was mixed with 1,500 KSL cells from IRF-2-/- mice and injected into lethally irradiated Ly5.1 mice. Engraftment analysis by peripheral blood chimerism (CD45.2 versus CD45.1 x CD45.2) at 4, 8 and 12 weeks after transplantation showed a profound deficit in wild-type recipient marrow, as peripheral blood elements derived from IRF-2-/- HSCs were progressively lost in favor of wild-type cells (Fig. 8A). Many more KSL cells could be engrafted into recipient mice if HSCs existed in the KSL fraction. However, a 24-fold larger number of cells also failed to rescue the recipients (Fig. 8B). When cells from the lineage-CD48 fraction were injected into recipients, no engraftment was shown in the recipients injected with cells from IRF-2-/- mice (Fig. 8C). The proportions of cells in the lineage-CD48 fraction isolated from both wild-type and IRF-2-/- mice were very similar (3.4% versus 2.9%). However, the number of CD150-positive cells was much lower in the IRF-2-/- mice compared to the wild-type mice (Fig.1).

To examine whether HSCs existed in fractions other than the KSL fraction of bone marrow cells in IRF-2-/- mice, whole bone marrow cells were injected into recipient mice and noncompetitive transplant assays were also performed. Transplantation of 1x10^5 wild-type bone marrow cells is normally sufficient to rescue and fully repopulate the hematopoietic systems of all lethally irradiated recipients. This dose of cells from KO donors failed to rescue any recipients from lethal irradiation, indicating impaired self-renewal of HSCs in whole bone marrow cells derived from IRF-2-/- cells. A dose of 2 x 10^6 cells from IRF-2-/- mice could rescue recipients from lethal irradiation, although the engraftment efficiency was poorer than that of wild-type at 1 month after transplantation (Fig. 8D).
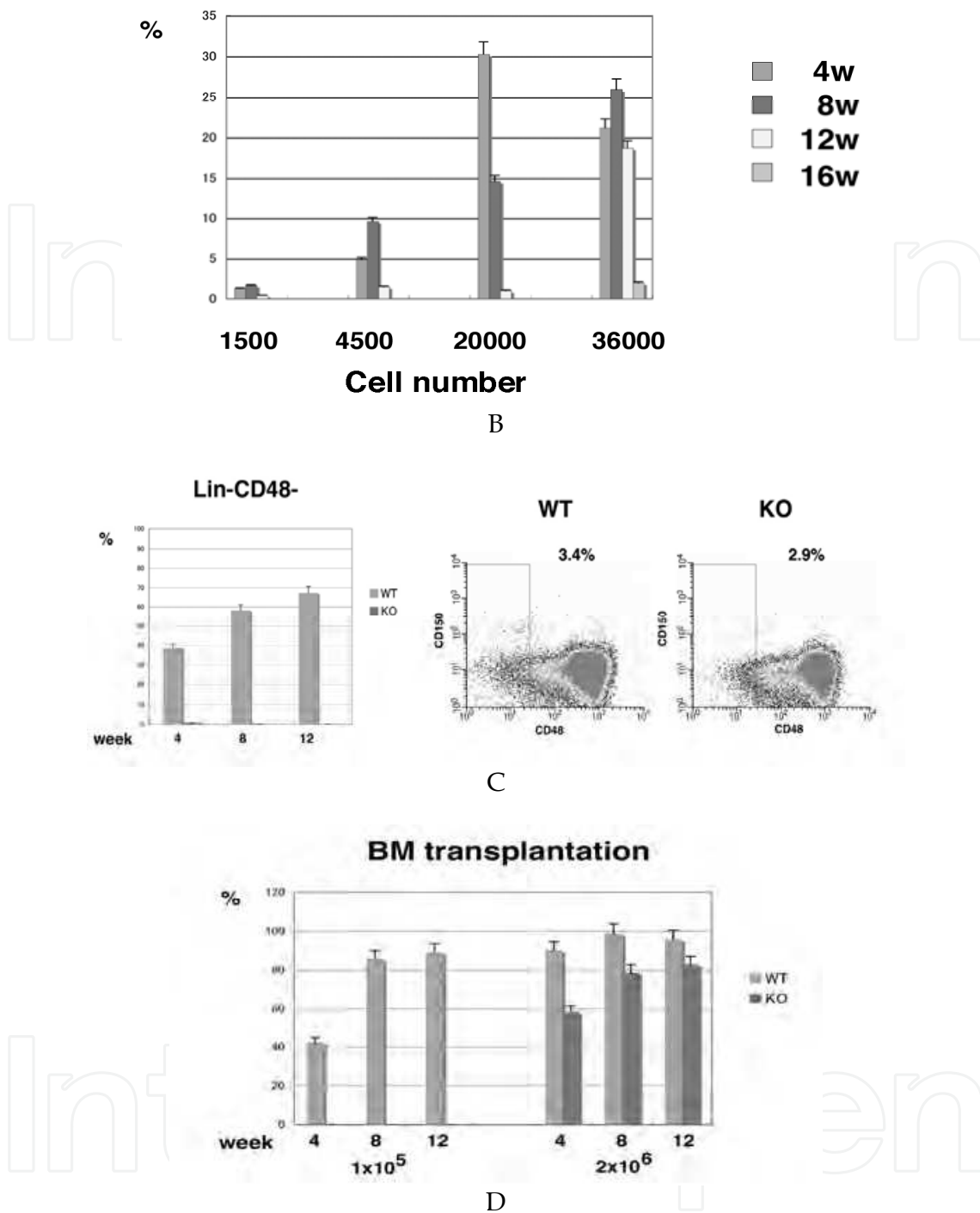

A

B

C

D
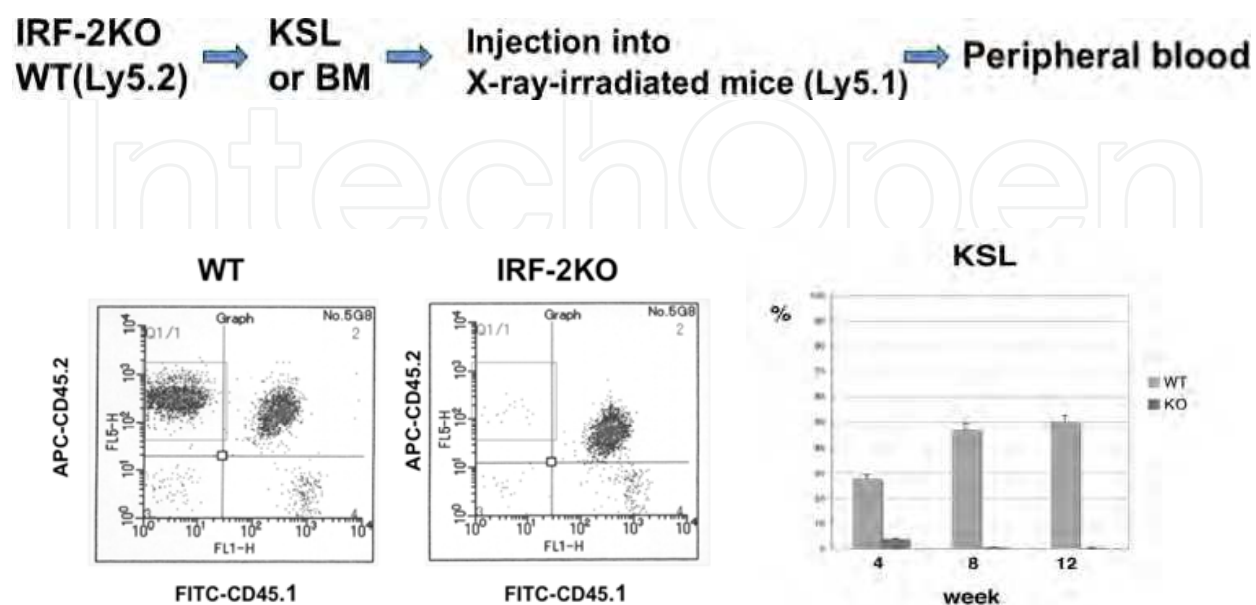
Fig. 8. Transplantation analysis. Cells in the KSL population were isolated from IRF-2-/-
mice (Ly5.2) and injected into X-irradiated mice. Peripheral blood cells were analyzed 4, 8
and 12 weeks after transplantation. (A) Percentage of donor-derived KSL cells (1500-cell
injection) in the blood. (B) Percentage of KSL derived from IRF-2-/- mice (injection of
indicated cell numbers) in the blood. (C) Percentage of Lin-CD48- cells from bone marrow of
wild-type and IRF-2-/- mice in the blood. (D) Percentage of donor-derived whole bone
marrow cells in the blood. Cells were grown in one of three mice injected with 2x10⁶ whole
bone marrow cells derived from IRF-2-/- mice. Representative data are shown from three
independent experiments.

An IRF-2-expressing retrovirus (Masumi et al. 2009) was transduced into the KSL fraction of IRF-2-deficient mouse bone marrow cells. When these IRF-2-expressing KSL cells were injected into lethally irradiated recipients, no sufficient rescue of engraftment was observed (data not shown). KSL cells from IRF-2-deficient mice may be distinct from those of wild-type mice. IRF-2 expression does not contribute to rescue the HSC function in KSL cells from IRF-2-/- mice in vivo.

IRF-2-/- mice were previously reported to be more sensitive to 5-fluorouracil (5-FU) than IRF-2+/- mice because of a progressive decrease in functional HSCs in IRF-2-/- mice (Sato et al., 2009). We treated both IRF-2-/- and wild-type mice weekly with 5-FU. Four of the IRF-2-/- mice died after the initial injection, but one mouse lived after three injections. We conclude that quiescent HSCs are present in whole bone marrow from IRF-2-/- mice, although the IRF-2-/- mice are more sensitive than wild-type (Fig. 9). As seen in Fig. 8D and Fig. 9, HSC-like cells, which may be isolated using cell surface markers distinct from KSL cells, are thought to be present in IRF-2-/-mice bone marrow cells (Fig. 10).
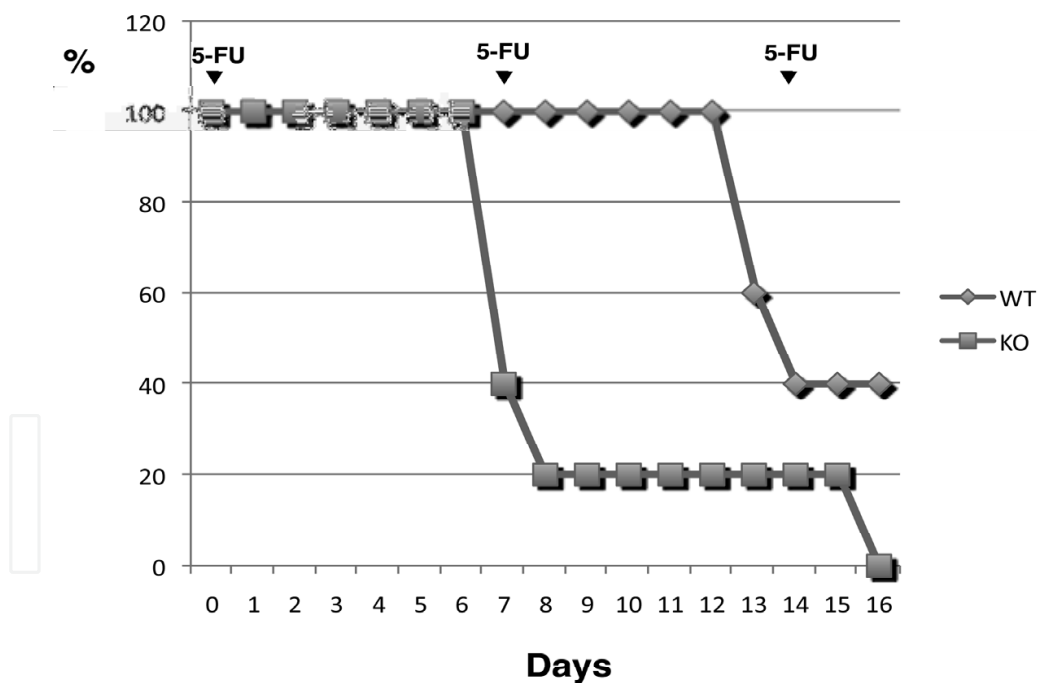

Fig. 9. Rate of survival (%) of wild-type and IRF-2-/- mice that were injected weekly with 150 mg/kg body weight of 5-FU (Sigma Chemical Co.); n= 5 for each group.
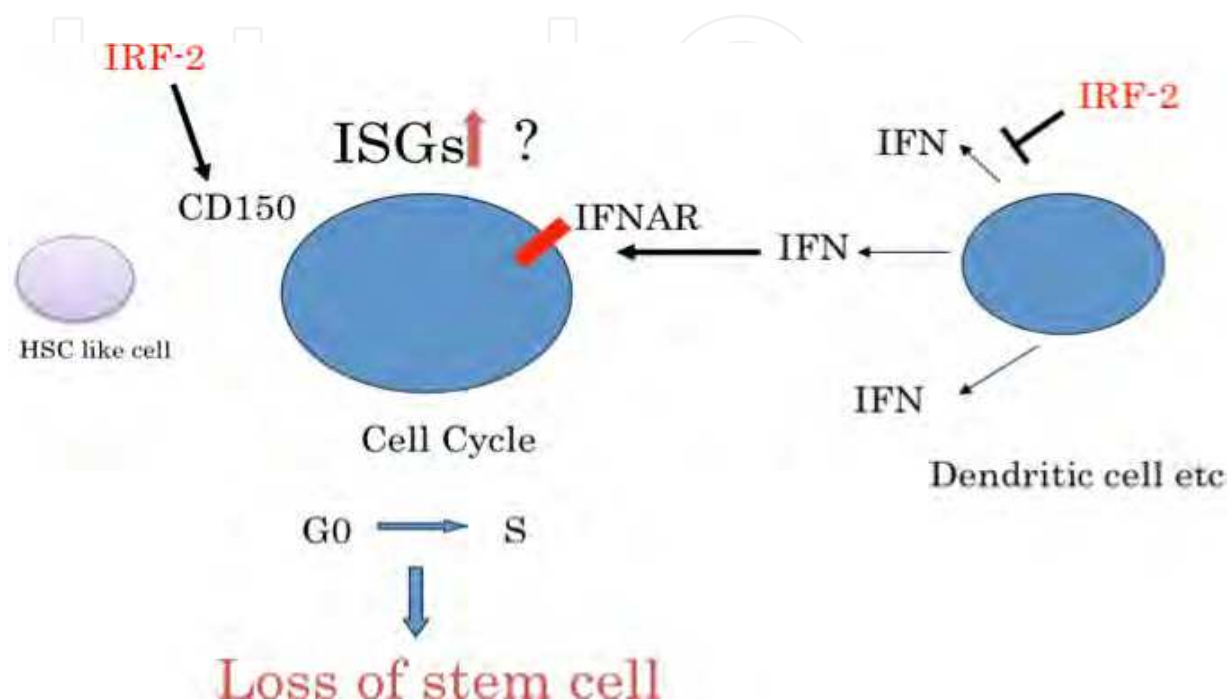
Fig. 10. HSC-like cells will be appeared in IRF-2-/- mice bone marrow cells (Refer to Fig.2C). IRF-2 may inhibit the down-regulation of CD150 gene expression by type I IFN.

2.2 Progenitors in bone marrow cells from IRF-2-/- mice

Next we analyzed the progenitor population in bone marrow cells from IRF-2-/- mice. Akashi et al proposed the model of major hematopoietic maturation pathways from HSCs (Akashi et al. 2000). According to his proposal, granulocyte/macrophage lineage progenitor (GMP), megakaryocyte/erythrocyte lineage progenitor (MEP), and common myeloid progenitor (CMP) and common lymphoid progenitor (CLP) were isolated from wild-type and IRF-2-deficient mice bone marrow. The frequencies of MEPs (Lin-ckit^{lo}sca-1-FcRg^{lo}CD34-) and CMPs (Lin-ckit^{lo}sca-1-FcRg^{lo}CD34+) were slightly decreased in IRF-2-deficient mice compared to wild-type mice. By contrast, GMPs (Lin-ckit^{lo}sca-1-FcRg^{high}CD34+) were slightly increased in IRF-2-deficient mice (Fig. 11A). The frequency of the CLP compartment in IRF-2-/- mouse bone marrow cells was lower than that from wild-type bone marrow (Fig. 11B).
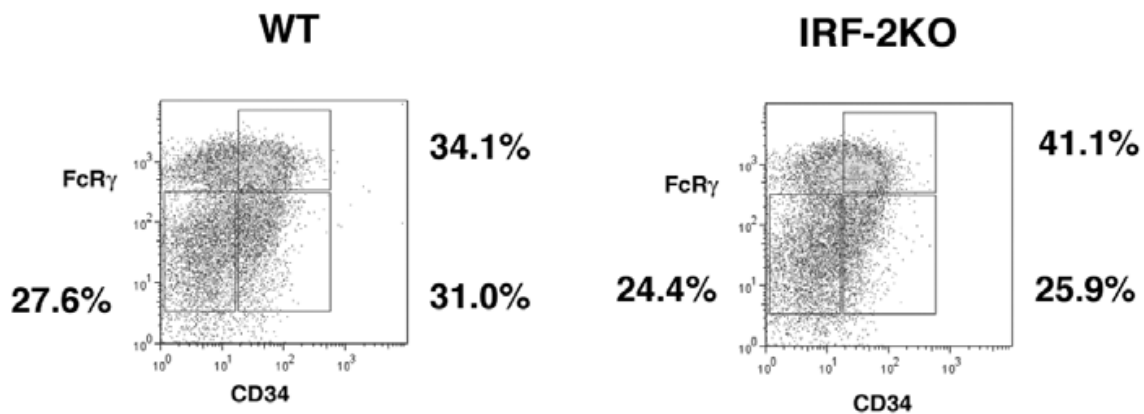
A
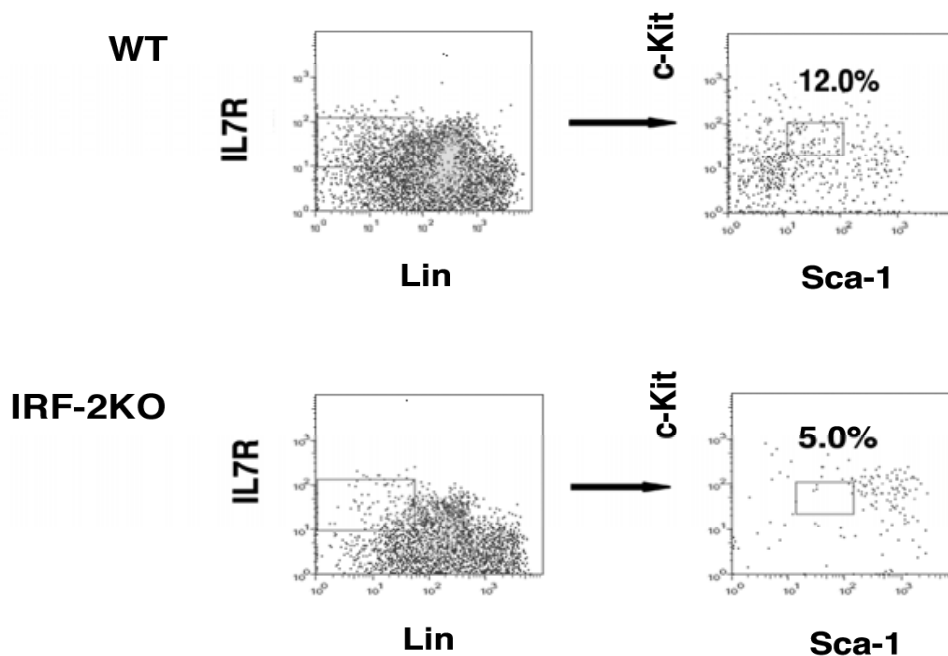
B

Fig. 11. (A) Lineage relationships among the myeloid progenitor subsets. MEPs (Lin-ckit^{lo}sca-1-FcRg^{lo}CD34-), CMPs (Lin-ckit^{lo}sca-1-FcRg^{lo}CD34+), and GMPs (Lin-ckit^{lo}sca-1-FcRg^{high}CD34+) are indicated. (B) Common lymphoid progenitors in bone marrow from IRF-2-/- mice.

2.2.1 Mouse colony-forming cell (CFC) assays with bone marrow derived from IRF-2-/- mice

The size of the GMP population from IRF-2-/- mice was higher than that from wild-type. We analyzed bone marrow and spleen cells from IRF-2-/- mice with CFU-GM assay. Colony numbers were higher in the bone marrow and spleen cells from IRF-2-/- mice, likely to the KSL population (Fig. 12 and Fig. 4).
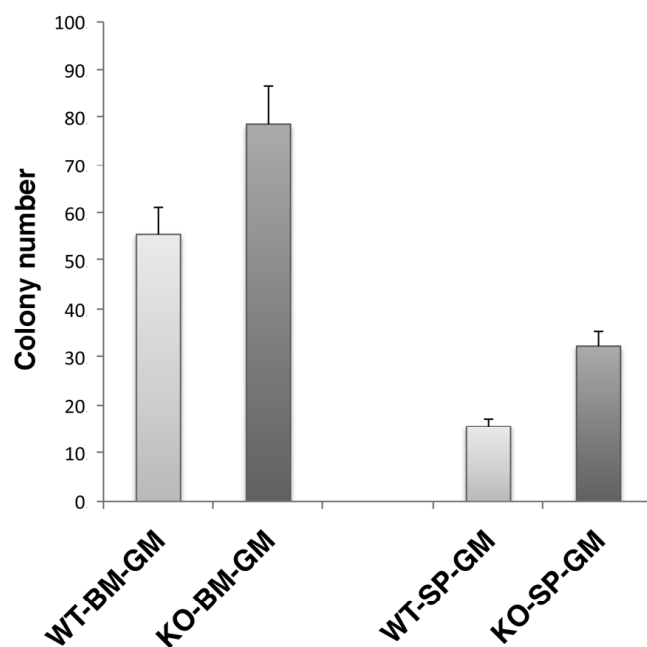
Fig. 12. Clonogenic progenitor assay with bone marrow and spleen cells from wild-type and IRF-2-/- mice. Assays performed as described in methods for Fig. 4. BM: bone marrow; SP: spleen

2.3 IRF-2 is required for bone marrow lymphopoiesis

To confirm the direct effect of IRF-2 deficiency in KSL cells, complementary DNA microarray analysis was performed on sorted Lin-c-Kit+Sca-1+ cells from bone marrow of wild-type and IRF-2-/- mice. This analysis showed that the up-regulated genes included IFN-inducible genes, such as Ly6s and Ifits, and the pre-B lymphocyte gene family (data not shown). As shown in Fig.13, when bone marrow B cell progenitors are analyzed, severe reduction in the frequency of mature IgM+ B cells was detected in the IRF-2-/- mice; and an enhanced frequency of pre-pro-B cells was detected in young IRF-2-deficient mice. These defects were correlated with the KSL array data (not shown). These data indicate a requirement for IRF-2 in maintaining bone marrow B homeostasis and B cell differentiation.
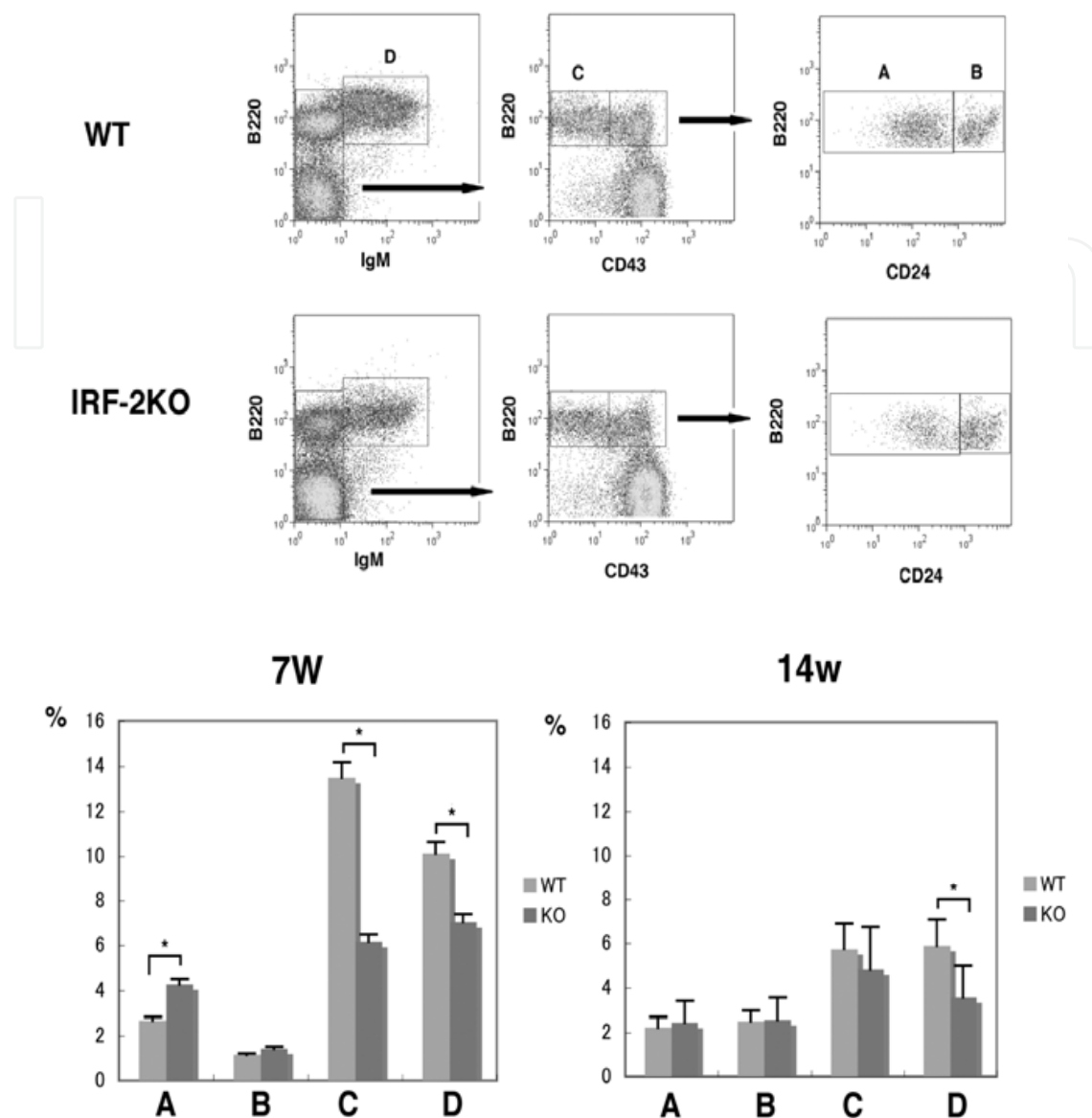
A: pre pro-B, B: pro-B, C: pre-B, D: Mature B

Fig. 13. Loss of IRF-2 in hematopoietic cells results in impaired B cell homeostasis. Top panel: FACS analysis of frequencies of B-cell subsets in bone marrow of IRF-2-/- and age-matched littermate control mice, for expression of B220, IgM, CD43 and CD24. Bottom panel: Four to five (each) young (7W) and old (14W) mice were analyzed. The frequency of mature B220+IgM+B cells (D) was significantly reduced in both young and old IRF-2-/- mice. Young, but not old, IRF-2-/- mice exhibited significant reductions in pre-B fractions (C) and increases in pre pro-B fractions (A).

Fluorescence-activated cell sorting (FACS) analysis of peripheral blood indicated that any significant differences in lineage between wild-type and IRF-2-/- mice were not observed (data not shown). However, an increase in Gr1+Mac1+ neutrophils and a decrease in B220+ cells were detected in the bone marrow and spleens IRF-2-/- mice (Fig.14). These lineage populations almost reflect to that of progenitors in IRF-2-/-mice (Fig.11).
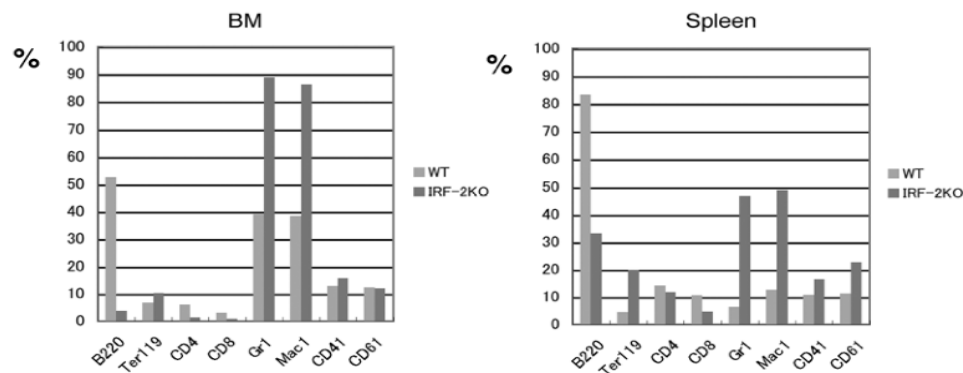
Fig. 14. Lineage populations in IRF-2-/- mouse bone marrow and spleens. Mononuclear cells from mouse bone marrow (BM) or spleen cells were stained with each antibody conjugated to PE and analyzed by FACS. Percentage indicates relative counts per whole mononuclear cells in each surface marker analysis.

2.4 Effect of type I IFN in IRF-2-/- mice

To assess the role of IRF-2 in the regulation of type I IFN signaling, we analyzed gene expression in IRF-2-/- mice compared to IRF-2-/- IFNAR-/- dKO mice, which do not respond to type I IFN. Sca-1 gene expression was enhanced in the bone marrow of IRF-2-/- mice, but not in IRF-2-/-/IFNAR-/- dKO mice. However, reduced expression of IFN-γ in IRF-2-/- mice was not rescued in IRF-2-/-IFNAR-/- dKO mice. Sca-1 expression is regulated by IRF-2 and the type I IFN response. However, IFN-γ may be regulated by IRF-2, independent of the type I IFN response. Arakura et al. reported the up-regulation of IFN-γ resulting from aberrant IFN-α/IFN-β responses in abdominal skin from IRF-2-/- mice (Arakura et al., 2007). In the bone marrow and KSL cells of our IRF-2-/- mice, IFN-γ expression was extremely low and the defect in IFN-α/IFN-β signaling did not rescue the expression. IFN-α/IFN-β expression was not enhanced in bone marrow and KSL cells in IRF-2-/- mice in the absence of stimulation (Fig. 8).

IFN-γ expression decreased in the bone marrow of both IRF-2-/- mice and IRF-2-/- IFNAR-/-dKO mice (Fig. 8B and Fig. 15). IFN-γ reduction is independent of type I IFN signaling in bone marrow cells from IRF-2-/- mice. IRF-2 may regulate IFN-γ gene expression through its promoter or other factors.
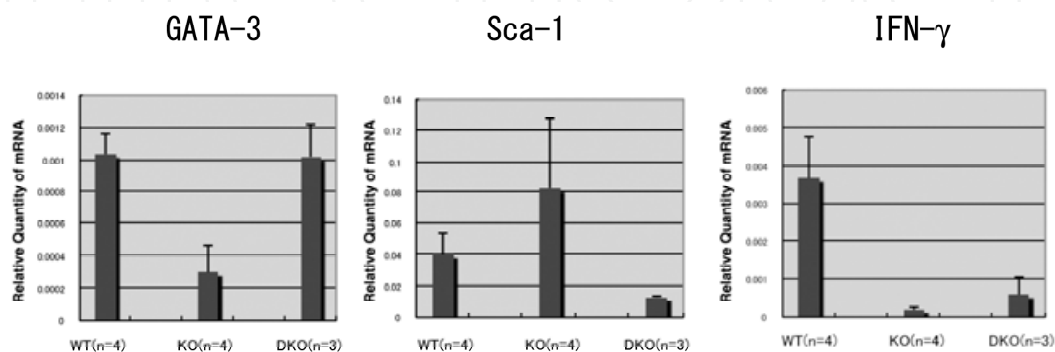

Fig. 15. Real-time PCR analysis for Sca-1, IFN-γ and GATA-3 gene expression in bone marrow cells from wild-type (WT), IRF-2-/- (KO)and IRF-2-/- IFNAR-/- dKO (DKO)mice.

A FACS analysis revealed the reduction of the CD150 surface marker in IRF-2-/- mice. Using real-time RT-PCR for expression of the CD150 gene in IRF-2-deficent mouse bone marrow, we demonstrated a profound decrease of CD150 gene expression (Fig. 16). However, the CD150 expression level in IRF-2-/- IFNAR-/- dKO bone marrow was comparable to wild-type mice. These results indicate that CD150 expression is regulated by the type I IFN response and is transcriptionally regulated in IRF-2-/-mice (Fig. 16). Sato et al. reported that the HSC population, including CD150-positive cells, was reduced through the induction of HSC proliferation by type I IFN signaling. However, we revealed another mechanism in which IRF-2 or type I IFN signaling directly mediated CD150 gene expression. In contrast, more depressed expression of Sca-1 was detected in IRF-2-/-IFNAR-dKO mice compared to that in wild type, supporting that Sca-1 expression was regulated by the type I IFN system (Fig. 16).
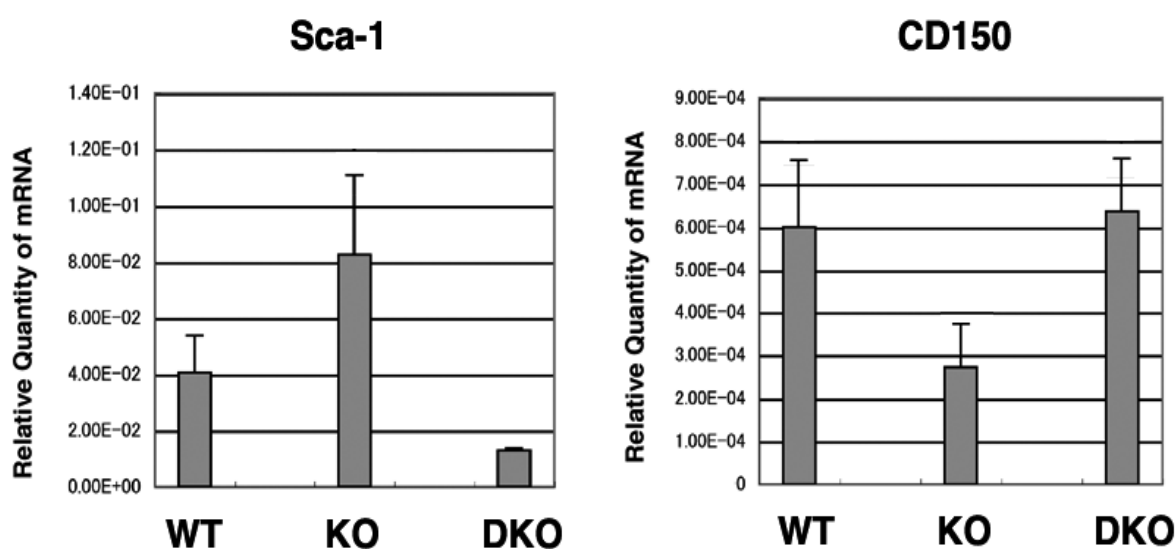

Fig. 16. Real-time PCR analysis for CD150 in bone marrow cells from wild-type (WT), IRF-2-/- (KO) and IRF-2-/- IFNAR-/- dKO (DKO) mice.

2.5 IRF-2 interaction with transcription factors in KSL

We investigated IRF-2-interacting transcription factors that are associated with hematopoiesis. TF(Transcriptional factor)-TF analysis indicates that IRF-2 associates GATA-1/2. However, we did not observe any difference of GATA-1 (data not shown) and GATA-2 (Fig.7A) expressions between wild-type and IRF-2-/- mice mice by KSL array data and real-time PCR analysis. We performed in vitro protein interaction analysis using 293T culture cells. IRF-2 interacted with GATA-2, but not GATA-1, when Flag-tagged IRF-2 and HA-tagged GATAs were transfected into 293T cells. To examine which region of IRF-2 interacts with GATA-2, IRF-2 DNA binding domain (DBD) and IRF-2 without DBD were incubated with several deletion constructs of GATA-2. The IRF-2 DNA-binding domain associated with GATA-2, specifically the N-terminal transcription activation domain in 293T cells (Fig. 17).
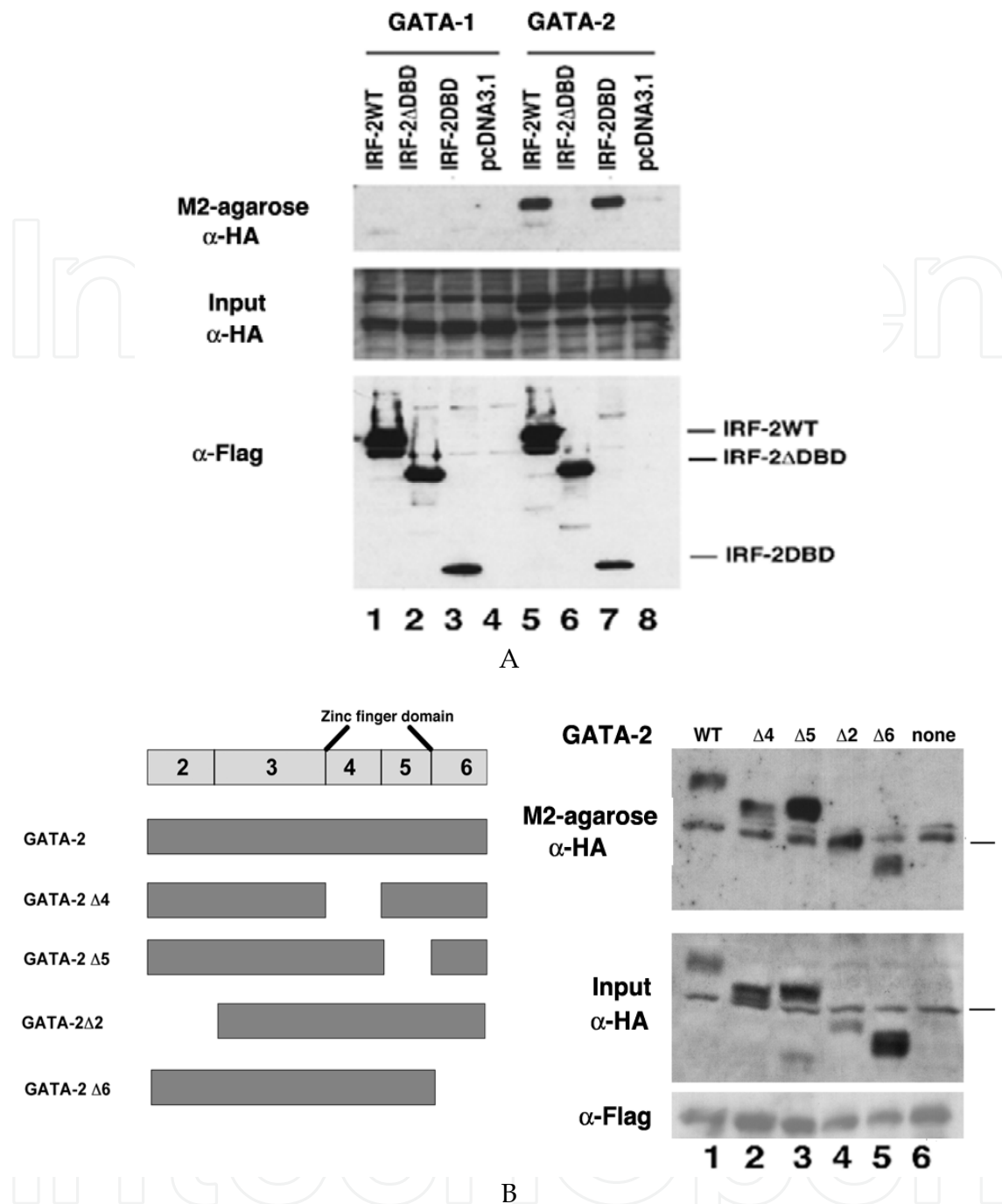
A

B

Fig. 17. IRF-2 interacts with GATA-2, but not GATA-1. (A) Flag-tagged IRF-2, Flag-tagged-IRF-2 DNA binding domain (IRF-2DBD) or Flag-tagged IRF-2 without DBD (IRF-2ΔDBD) and HA-tagged GATA-1 or HA-tagged GATA-2 were transfected to 293T cells. Cell lysate were incubated with M2-agarose, and agarose were washed and eluted with Flag peptide solution. Eluted fraction were electrophoresed and Western blot analysis was performed. (B) Protein structure of mouse GATA-2 and its mutants (GATA-2Δ4, GATA-2Δ5, GATA-2Δ2 and GATA-2Δ6) were shown (left) and numbers indicate exons of GATA-2. Each exon deletion mutants tagged with HA was incubated with Flag-tagged IRF-2. Exon2 (transcriptional activation domain) in GATA-2 has high affinity for binding with IRF-2 (right). – indicates non-specific bands.

We found that GATA-3 gene expression was decreased in IRF-2-deficient bone marrow and KSL cells by KSL array data and real-time PCR analysis (Fig. 15). GATA-3 gene expression was comparable to that in IRF-2-/- IFNAR-/- dKO mice, suggesting that the type I IFN response affects GATA-3 expression in IRF-2-/- mice. To examine GATA-3 interacts with IRF-2, Flag-tagged IRF-2 and myc-GATA-3 expression vectors were transfected to 293T cells. We show that GATA-3 interacted with IRF-2 in 293T cells (Fig. 18).
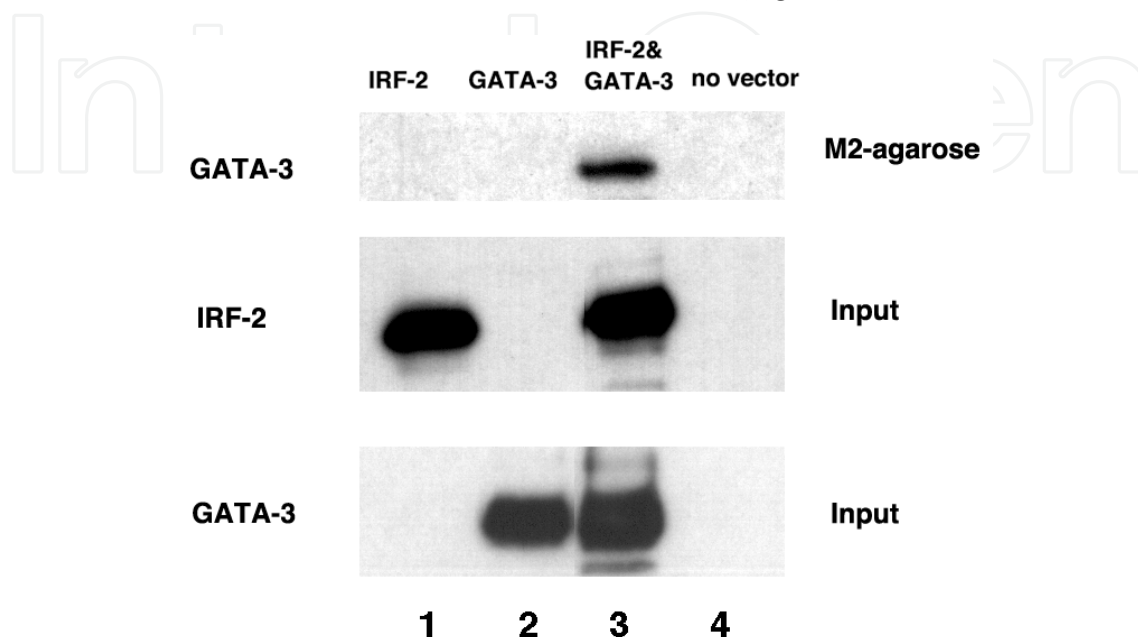

Fig. 18. IRF-2 interacts with GATA-3. Flag-tagged IRF-2 and Myc-tagged GATA-3 were transfected to 293T cells. Western blot analysis was performed as described in Fig.17.

In our investigation, GATA-2 and GATA-3 associated with IRF-2 in the in vitro cell culture system. GATA-2 is expressed abundantly in the mouse HSC population and is necessary for hematopoietic differentiation (Kitajima et al. 2006). GATA-1 is expressed in megakaryocyte/erythrocyte precursors (MEP) and their progenitors, and GATA-3 is expressed in common lymphoid precursors (CLPs) and T cells. There are very few reports regarding GATA-3 expression in HSCs, although we found that the GATA-3 expression level changed in IRF-2-deficient bone marrow and KSL in microarray and real time PCR analysis. Previous reports have shown that forced GATA-3 expression into mouse HSCs induces differentiation toward erythrocytes and megakaryocytes (Chen and Zhang 2001). As indicated by our investigation, GATA-3 may be important for the maintenance of HSC cooperation with IRF-2 or IFN signaling. These findings indicate that the interactions between IRF-2 and the GATAs are required to maintain HSC function in mouse bone marrow cells. Interactions between GATA-2 and GATA-3 with IRF-2 in HSCs should be clarified in future experiments.

3. Conclusion

IRF-2 exists high in the mouse bone marrow HSC population and helps maintain the protective immune response, which responds to viral or bacterial infection and inflammation, resulting in IFN producing system. HSCs are essential for the production of immune cells, such as myeloid or lymphoid cells. Recently, interferon treatment has been

reported to be a target for cancer stem cells. Under chronic interferon stimulation, such as in an IRF-2-deficient condition, not only HSCs, but also cancer stem cells can be activated. Blocking the IRF-2 function may induce to eliminate of cancer stem cell through IFN signaling. However, the possibility of the presence of HSC-like cells in IRF-2 deficient mice cell population necessitates further investigation because IRF-2 in part regulates HSC populations independent of the type I IFN system in an another possible mechanism.

4. Acknowledgments

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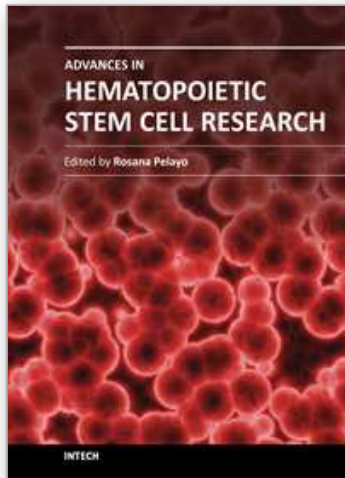
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