IRF-4 Suppresses BCR/ABL Transformation of Myeloid Cells in a DNA Binding-independent Manner*

Seung-Hee Jo and Ruibao Ren
From the Rosenstiel Basic Medical Sciences Research Center and Department of Biology, Brandeis University, Waltham, Massachusetts 02454

Background: IRF-4 functions both as an oncoprotein and as a tumor suppressor in different cell context.

Results: We found that IRF-4 inhibits BCR/ABL transformation of myeloid cells independent of its DNA binding and nuclear localization.

Conclusion: The oncogenic and tumor suppressor functions of IRF-4 involve distinct pathways.

Significance: The studies help to develop improved therapies for malignancies involving IRF-4.

Interferon regulatory factor 4 (IRF-4)2 is essential for B and T cell development and immune response regulation, and has both nuclear and cytoplasmic functions. IRF-4 was originally identified as a proto-oncogene resulting from a t(6;14) chromosomal translocation in multiple myeloma and its expression was shown to be essential for multiple myeloma cell survival. However, we have previously shown that IRF-4 functions as a tumor suppressor in the myeloid lineage and in early stages of B cell development. In this study, we found that IRF-4 suppresses BCR/ABL transformation of myeloid cells. To gain insight into the molecular pathways that mediate IRF-4 tumor suppressor function, we performed a structure-function analysis of IRF-4 as a suppressor of BCR/ABL transformation. We found that the DNA binding domain deletion mutant of IRF-4, which is localized only in the cytoplasm, is still able to inhibit BCR/ABL transformation of myeloid cells. IRF-4 also functions as a tumor suppressor in bone marrow cells deficient in MyD88, an IRF-4-interacting protein found in the cytoplasm. However, IRF-4 tumor suppressor activity is lost in IRF association domain (IAD) deletion mutants. These results demonstrate that IRF-4 suppresses BCR/ABL transformation by a novel cytoplasmic function involving its IAD domain.

Interferon regulatory factor 4 (IRF-4)2 plays a critical role in B, T, and dendritic cell development as well as in immune response regulation (1, 2). IRF-4 was also originally identified as a proto-oncogene resulting from a t(6;14)(p25;q32) chromosomal translocation in multiple myeloma (3). In addition, IRF-4 expression has been shown to be an unfavorable prognostic factor in B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma (4). Recent studies have shown that expression of IRF-4 is essential for the maintenance of multiple myeloma cells (5), making IRF-4 an attractive target for the development of therapies for multiple myeloma.

In addition to its role as a transcription regulator, IRF-4 was recently found to function as a negative regulator of Toll-like receptor (TLR) signaling in the cytoplasm by interacting with the myeloid differentiation primary response protein 88 (MyD88), a common adapter protein for all TLRs except TLR3 (6, 7). TLR signaling plays a central role in the innate immunity against microbial pathogenesis by inducing the expression of immunity and pro-inflammatory genes (8). In addition, TLRs are expressed in a wide variety of tumors and the activation of TLR signaling promotes tumor cell growth and survival (9–11). IRF-4 binds to MyD88 on the same site bound by IRF-5, whose activation by interacting with MyD88 is essential for the induction of pro-inflammatory cytokines in response to TLR stimulation (12), thereby attenuating IRF-5 activation. Consistently, TLR-induced cytokine production is significantly increased in peritoneal macrophages from IRF-4-deficient mice (6, 7). In addition, the activation of NF-κB was also enhanced in response to TLR stimulation in IRF-4-deficient macrophages (6).

Though IRF-4 is also expressed in myeloid cells, its function in the myeloid lineage is not known. The closely related IRF family member IRF-8 plays an important role in myelopoiesis and in anti-viral immunity (13). IRF-8-deficient mice spontaneously develop a chronic myelogenous leukemia (CML)-like disease, indicating that IRF-8 functions as a tumor suppressor (14). Human CML is a myeloproliferative disease characterized by the underlying t(9;22)(q34;q11) reciprocal chromosome translocation resulting in what is known as the Philadelphia chromosome, which leads to the creation and expression of the fusion gene product BCR/ABL, a deregulated tyrosine kinase (15). We have shown that IRF-8 is down-regulated in a BCR/ABL-induced murine CML and that forced overexpression of IRF-8 in this model represses the resulting myeloproliferative disorder and prolongs survival (16). In addition, IRF-8 has overlapping functions with IRF-4 in early B cell development. Both proteins can interact with tran-
scription factors PU.1 and E2A at the Ig and H9260 and H9261 light chain enhancer regions (17) and both regulate Ikaros and Aiolos expression (18). Consistently, mice deficient in both IRF-4 and IRF-8 show a block in B cell development at the pre-B to immature B transition and display an accumulation of cycling pre B cells (19).

In contrast to its oncogenic function in mature lymphocytes, expression of IRF-4 is down-regulated in BCR/ABL positive CML and acute B-lymphoblastic leukemia (B-ALL) (20–22). In CML, lower expression of IRF-4, like IRF-8, is correlated with a higher burden of pretreatment risk factors and a lower response rate to treatment with IFN-α, the standard treatment for CML before the advent of tyrosine kinase inhibitors such as imatinib myselate (21, 23). These data suggest that IRF-4 may have different functions in different cell contexts. Indeed, we have recently shown that IRF-4 and IRF-8 deficiencies can cooperate in the development of both myeloid and lymphoid tumors (24) and that IRF-4 deficiency facilitates the development of BCR/ABL-induced B-ALL, while forced expression of IRF-4 potently suppresses the pathogenesis of BCR/ABL-induced B-ALL (25). These findings demonstrate that IRF-4 functions as a tumor suppressor in the myeloid lineage and in early stages of B cell development.

The finding of IRF-4 functioning as a tumor suppressor raises caution for developing therapies aiming to down-regulate IRF-4. Further studies on the mechanisms by which IRF-4 functions as an oncoprotein and as a tumor suppressor are necessary to develop improved therapies for malignancies involving IRF-4. IRF-4 contains multiple functional domains/motifs and localizes in both the nucleus and the cytoplasm. To gain insight into the molecular pathways that mediate IRF-4 tumor suppressor function, we performed a structure-function analysis of IRF-4 in suppressing BCR/ABL transformation in myeloid cells. We found that IRF-4 suppresses BCR/ABL transformation independent of its DNA binding and nuclear localization, and in a MyD88-independent manner. The IRF-association domain of IRF-4, on the other hand, is essential for its tumor suppressor function.

**EXPERIMENTAL PROCEDURES**

*Mice—IRF-4 knock-out (KO) mice in C57BL/6 background were bred and genotyped as described previously (26). MyD88 KO mice in C57BL/6 background were kindly provided by Dr. Douglas Golenbock at the University of Massachusetts Medical School. Wild-type C57BL/6 mice were purchased from Taconic Farms (Germantown, NY). Mice used in this study were housed in the Association for Assessment and Accreditation of Laboratory Animal Care International-accredited Foster Animal Research Facility in Brandeis University and procedures were approved by the Institutional Animal Care and Use Committee of Brandeis University.*
DNA Constructs—Production of the murine stem cell virus (MSCV)-BCR/ABL-GFP-IRES-2xmyc-IRF-4, MSCV-BCR/ABL-GFP-IRES-2xmyc-Neo and MSCV-GFP-IRES-2xmyc-IRF-4 were described previously (25). Deletion mutants were prepared by PCR reaction using Pfu polymerase (Stratagene, La Jolla, CA). Construction of the IRF-4 DNA binding mutant (R98C99A) and the PU.1 interacting mutant (AS397) has been previously described (27). The cDNA of mouse MyD88 was amplified by PCR reaction using plasmid 13092 obtained from Addgene as a template. These PCR products were subcloned into the NotI and ClaI sites in-frame with an N-terminal Myc tag in MSCV-BCR/ABL-GFP-IRES-2xmyc-Neo and MSCV-GFP-IRES-2xmyc-IRF-4 were described previously (25). Deletion mutants were prepared by PCR reaction using Pfu polymerase (Stratagene, La Jolla, CA). Construction of the IRF-4 DNA binding mutant (R98C99A) and the PU.1 interacting mutant (AS397) has been previously described (27). The cDNA of mouse MyD88 was amplified by PCR reaction using plasmid 13092 obtained from Addgene as a template. These PCR products were subcloned into the NotI and ClaI sites in-frame with an N-terminal Myc tag in MSCV-BCR/ABL-GFP-IRES-2xmyc-Neo and MSCV-GFP-IRES-2xmyc-IRF-4. All IRF-4 deletion mutants were confirmed by sequencing. The control vectors, MSCV-GFP-IRES and the MSCV-IRES-GFP, were made as described previously (25).

Cell Culture and Retrovirus Production—Bosc23 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Sigma), 50 μg/ml gentamicin (Invitrogen, Grand Island, NY). NIH3T3 cells were maintained in DMEM containing 10% donor bovine serum (DBS, Invitrogen), 50 μg/ml gentamicin. Retroviruses were produced as described previously (28). Briefly, MSCV constructs were transfected into Bosc23 cells by the calcium phosphate precipitation method. Two days after transfection, retrovirus-containing supernatants were recovered and centrifuged. The viral titer was calculated based on the percentage of GFP-expressing NIH3T3 cells as described previously (29). NIH3T3 cell lines stably expressing both GFP-fused BCR/ABL and Myc-tagged IRF-4 mutant, or the control GFP were generated by retroviral infection as described (29). All GFP-positive cell lines were sorted by fluorescence-activated cell sorting (FACS) using a FACSArray (BD Biosciences, San Jose, CA). These cell lines were maintained and confirmed to express GFP by FACS analysis using a FlowJo software (TreeStar, San Carlos, CA).

**FIGURE 2.** IRF-4 suppresses BCR/ABL-stimulated bone marrow colony formation. A and B, bone marrow cells from 5-FU treated mice were infected with titer-matched retroviruses containing BCR/ABL-GFP + Neo, BCR/ABL-GFP + IRF-4, BCR/ABL-GFP + IRF-8, or GFP, then 5 × 10⁴ cells were plated in soft agar media (triplicates) in the absence of cytokines. BCR/ABL-GFP + Neo infected culture had significantly larger (A) and more (B) colonies than cultures infected with BCR/ABL-GFP + IRF-4 (p = 0.003) or BCR/ABL-GFP + IRF-8 (p = 0.018). BCR/ABL-GFP + IRF-4 had also significantly fewer colonies than BCR/ABL-GFP + IRF-8 (p = 0.011). C and D, bone marrow cells from 5-FU treated mice were either transduced with the GFP control retroviruses or co-transduced with the same amount of BCR/ABL-GFP retroviruses with various ratio of IRF-4 and GFP retroviruses as indicated, then 2 × 10⁵ cells were plated in soft agar media in the absence of cytokines. Significantly fewer colonies were found in cultures with increasing quantity of IRF-4 retroviruses.
Immunoblotting—32D cells (1 × 10⁶) were infected with retrovirus containing BCR/ABL-GFP + Neo, BCR/ABL-GFP + IRF-4, BCR/ABL-GFP + IRF-8, and GFP alone as described (29). GFP + 32D cells were sorted to a purity of ~99% by FACS, and maintained in DMEM containing WEHI-contained medium. Cell lysates were separated on 6–18% polyacrylamide gradient gels, transferred to nitrocellulose membranes, and blotted with anti-ABL monoclonal antibody Ab3, anti-myc tag monoclonal antibody clone 9E10, or anti-phosphotyrosine monoclonal antibody clone 4G10 (Upstate Biotechnology, Lake Placid, NY). The relative expressions of IRF-4 and IRF-8 were quantified using NIH image software (NIH, Bethesda, MD).

Cytoplasmic and nuclear extracts were prepared from NIH3T3 cell lines expressing both GFP-fused BCR/ABL and Myc-tagged IRF-4 mutants by NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce Biotechnology) according to the manufacturer’s instruction. The concentrations of cytoplasmic and nuclear extracts were measured by Bradford assay. The protein samples were separated by 6–18% or 18% polyacrylamide gradient gels, then transferred to nitrocellulose membranes in CAPS buffer. The membranes were probed with anti-IRF-4 (sc-6059, Santa Cruz Biotechnology, Inc., Santa Cruz, CA), anti-Myc-Tag (9E10; Cell Signaling Technologies, Beverly, MA), and HRP-labeled rabbit anti-goat IgG or goat anti-mouse IgG (Pierce Biotechnology). Detection was carried out with Super Signal West Femto Chemiluminescence Reagents (Pierce Biotechnology). Blots were stripped and re-probed with nucleus-specific anti-Lamin B and cytoplasm-specific anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibodies (sc-6216 and sc-47724; Santa Cruz Biotechnology).

Bone Marrow Colony Assays—Bone marrow (BM) colony assays were performed as described previously (16). 5-fluorouracil (5-FU)-injected BM cells were infected with retroviruses for 2 days as described above, and then 2 × 10⁵ to 2 × 10⁶ cells were plated in triplicate in DMEM, 20% FBS, 50 μg/ml gentamicin, and 0.3% bacto agar onto 1 ml of bottom agar containing DMEM, 10% FBS, 50 μg/ml gentamicin, and 0.6% bacto agar. Cells were incubated at 37 °C, and colonies were counted after 10 days.
DNA-binding Independent Tumor Suppressor Function of IRF-4

Statistical Analysis—The Student’s t test was used in statistical analysis of BCR/ABL-stimulated bone marrow colony assays.

RESULTS

IRF-4 Inhibits BCR/ABL-stimulated Bone Marrow Colony Formation—It has been shown that IRF-4 is down-regulated in CML (20, 21). We wondered whether IRF-4 could negatively regulate BCR/ABL transformation in myeloid cells. To address this question, we examined whether forced expression of IRF-4 could suppress BCR/ABL transformation. We first made retroviral constructs by inserting a BCR/ABL-GFP fusion with either IRF-4 or Neomycin resistance gene (Neo) into the murine stem cell virus (MSCV) vector as depicted in Fig. 1A. IRF-8 has been shown to inhibit BCR/ABL-induced bone marrow colony formation (16), and we thus included IRF-8 in this study as a control. Prior to conducting the transformation assay, we confirmed that BCR/ABL-GFP expression was similar for all these MSCV constructs in 32Dcl3 (32D) myeloid progenitor cells (Fig. 1B). In addition, phosphotyrosine levels were similar in BCR/ABL-GFP + Neo, BCR/ABL-GFP + IRF-4, and BCR/ABL-GFP + IRF-8 expressing 32D cells (Fig. 1C), indicating that coexpression of IRF-4 or IRF-8 does not interfere with the kinase activity of BCR/ABL.

We then compared the abilities of the above retroviruses to stimulate cytokine independent bone marrow cell growth in soft agar. As expected, BCR/ABL-GFP, but not the GFP control, stimulated the formation of myeloid colonies under the condition used in the experiment (Fig. 2 and data not shown). Cultures of bone marrow cells infected with BCR/ABL-GFP + IRF-4 or BCR/ABL-GFP + IRF-8, on the other hand, had smaller and significantly fewer colonies after 10 days compared with BCR/ABL-GFP + Neo infected cells (Fig. 2A and B). Interestingly, BCR/ABL-GFP + IRF-4-infected bone marrow cells formed significantly fewer colonies than BCR/ABL-GFP + IRF-8-infected cells (Fig. 2B). These data show that IRF-4, like IRF-8, suppresses BCR/ABL transformation of myeloid cells, and suggest that IRF-4 may be an even more potent inhibitor of BCR/ABL transformation than IRF-8.

To rule out the possibility that the reduced transforming potential of BCR/ABL-GFP + IRF-4 retrovirus is due to an artifact from the use of a bicistronic vector, we also performed bone marrow colony assay by co-transducing bone marrow cells with retroviral vectors expressing BCR/ABL and IRF-4 separately. In this experiment, the amount of BCR/ABL retroviruses was kept constant, while IRF-4 and GFP retroviruses with various ratios (0:6, 2:4, 4:2) were used to co-transduce the bone marrow cells with the BCR/ABL retroviruses (Fig. 2C and D). At 10 days after plating, significantly fewer colonies were observed with the increased ratio of IRF-4 to GFP viruses. This result confirms the tumor suppressor activity of IRF-4 in BCR/ABL-induced transformation. The bicistronic vectors were used in the following experiments.

IRF-4 Inhibits BCR/ABL Transformation in a DNA Binding Domain-independent Manner—IRF-4 contains multiple functional domains/motifs and has both nuclear and cytoplasmic functions. The DNA binding domain of IRF-4 has been shown to be essential for IRF-4 to regulate class switch recombination and immunoglobulin (Ig) secretion (30), but it is not required in the negative regulation of TLR signaling by IRF-4 (7). To identify the structure elements of IRF-4 that are involved in its function as an inhibitor of BCR/ABL transformation, we performed a structure/function analysis using a series of Myc-tagged IRF-4 mutants (Fig. 3A). To test whether the DNA binding ability is essential for IRF-4 to inhibit BCR/ABL-stimulated bone marrow colony formation, we generated two DNA binding domain (DBD) deletion mutants, as well as a mutant IRF-4 with R98/C99A point mutations, which abolish the DNA binding ability of IRF-4 (30). Interestingly, all these mutants showed a similar tumor suppressor activity as the wild-type IRF-4 (Fig. 3, C and D), indicating that the DNA binding ability of IRF-4 is not required for its tumor suppressor function.

It has been shown that the nuclear localization signal of IRF-4 is located in its DNA binding domain (7). Consistent with the previous finding, we found that the DBD deletion mutant (∆DBD) was detected only in the cytoplasm, whereas the wild-type IRF-4 and the R98/C99A IRF-4 mutants were localized to both the nucleus and the cytoplasm. The small amount of 134–410 mutant detected in the nucleus may be caused by cytoplasmic contamina-
tion during fractionation. These results suggest that IRF-4 suppresses BCR/ABL transformation of myeloid cells in a DNA binding-independent manner and that the tumor suppressor function of IRF-4 primarily involves its cytoplasmic activities.

**IRF-4 DNA Binding Domain Mutants Inhibit BCR/ABL Transformation in the Absence of the Endogenous IRF-4**—The transcription regulatory function of IRF-4 can be modulated by IRF-4-binding protein (IBP) and FKBP52, which were shown to interact with IRF-4 and inhibit IRF-4’s DNA binding (31, 32). It is possible that the DNA binding mutants of IRF-4 may sequestrate negative regulators, allowing the endogenous wild-type IRF-4 to suppress BCR/ABL transformation by regulating gene expression. To test this possibility, we performed a bone marrow colony formation assay using bone marrow cells from the IRF-4 KO mice. We found that the DBD deletion mutant of IRF-4 has the same ability as wild-type IRF-4 in suppressing BCR/ABL-stimulated bone marrow colony formation in the absence of endogenous IRF-4 (Fig. 4, A and B). Together, these data confirm that IRF-4 can inhibit BCR/ABL transformation in a DNA binding domain-independent manner.

**The IAD Is Essential for IRF-4 to Suppress BCR/ABL Transformation**—To further delineate which region of IRF-4 is involved in its tumor suppressor function, we analyzed more mutants of IRF-4. IRF-4 can regulate many genes by interacting with a transcription factor PU.1, which is important in normal myeloid and lymphoid development (1, 33, 34). To test the importance of the IRF-4/PU.1 interaction for IRF-4 tumor suppressor activity, we checked the tumor suppressor activities of PU.1 binding mutants of IRF-4, AS397 and 1–380 (27, 35). AS397 is an alanine substitution mutant that is unable to bind PU.1, and 1–380 has a C-terminal deletion including the PU.1 binding region (Fig. 5A). Interestingly, these two mutants showed similar levels of tumor suppressor activity as did wild-type IRF-4 (Fig. 5, C and D), suggesting that the IRF-4/PU.1 interaction is not essential for IRF-4 tumor suppressor activity. We next determined the importance of the IAD domain in IRF-4 tumor suppressor activity using four deletion mutants, 1–200, DBD, ΔIAD, and ΔPro+IAD (Fig. 5A). All of the mutants were localized to both the nucleus and the cytoplasm (Fig. 5B). Bone marrow colony formation assays showed that these four mutants failed to inhibit BCR/ABL transformation (Fig. 5, C and D). Collectively, these data suggest that the IAD domain of IRF-4 is essential for its tumor suppressor function.

**IRF-4 Inhibits BCR/ABL Transformation in a MyD88-independent Manner**—One of the proteins that IRF-4 interacts with in the cytoplasm through its IAD domain is MyD88. To test...
DNA-binding Independent Tumor Suppressor Function of IRF-4

A

GFP

B/A+Neo

B/A+IRF4

B/A+IRF4 ΔDBD

B

# colonies

0

20

40

60

80

100

120

Neo

IRF-4

IRF-4 ΔDBD

FIGURE 6. Wild-type and the DNA binding domain deletion mutant of IRF-4 suppress BCR/ABL transformation in MyD88-deficient bone marrow cells. Bone marrow cells from MyD88 KO mice were infected with retroviruses as indicated, then $S \times 10^5$ cells were plated in soft agar. Representative soft agar plates from bone marrow colony formation assays and the average number of colonies from triplicates are presented in A and B, respectively. *, $p < 0.0001$ for comparisons between retroviral constructs as indicated and BCR/ABL alone.

whether MyD88 is required for the tumor suppressor function of IRF-4, we performed a bone marrow colony formation assay using bone marrow cells from MyD88 KO mice. The result showed that expression of the wild-type or DBD deletion mutant of IRF-4 significantly inhibits BCR/ABL-stimulated bone marrow colony formation (Fig. 6, A and B). These experiments demonstrate that IRF-4 functions as a tumor suppressor in a MyD88-independent manner.

DISCUSSION

In this study, we demonstrated that IRF-4 inhibits BCR/ABL-stimulated cytokine-independent myeloid colony formation in a DNA binding-independent manner and that the IAD domain of IRF-4 is critical for its tumor suppressor activity. The tumor suppressor function of IRF-4 involves its cytoplasmic activities, but its cytoplasmic-interacting protein MyD88 is not required for IRF-4 to suppress BCR/ABL transformation.

IRF-4 not only regulates genes that play crucial roles in normal hematopoietic cell development but is also involved with many genes regulating cell proliferation and survival in multiple myeloma (2). In particular, IRF-4 directly regulates the expression of MYC, which is essential for multiple myeloma cell survival (5). As such, IRF-4 acts as an oncogene primarily through its transcription-regulation function. However, the function of IRF-4 in suppressing transformation of myeloid cells by BCR/ABL is independent of DNA binding and involves its cytoplasmic function. The results indicate that the oncogenic and tumor suppressor functions of IRF-4 involve distinct pathways.

IRF-4 interacts with a number of transcription factors. One possible mechanism by which the DNA binding domain mutants of IRF-4 inhibit BCR/ABL transformation is that mutant IRF-4 sequestrates transcription factors that are critical for cell growth/survival. However, the interactions between IRF-4 and its transcription partners are weak and their stable interaction relies on DNA binding (1, 27). As such, the DNA binding domain mutants of IRF-4 are unlikely to function as dominant negative regulators.

IRF-4 localizes in both the nucleus and the cytoplasm. It is not clear how IRF-4 functions as a tumor suppressor in the cytoplasm. The general TLR signaling adapter, MyD88, has been shown to play critical roles in the development of several types of cancer (11). MyD88 deficiency leads to reduced growth of colon cancer in a APCmin+ mouse model (36), and MyD88-dependent activation of ERK promotes tumorigenesis (37). Because MyD88 is one of the IRF-4 interacting proteins in the cytoplasm, we thought that the negative regulation of TLR signaling by IRF-4, through the IRF-4/MyD88 interaction, might contribute to its tumor suppressing activity. However, we found that IRF-4 and the DNA binding domain deletion mutant of IRF-4 still suppress BCR/ABL transformation in MyD88-deficient bone marrow cells. These results suggest that IRF-4 suppresses BCR/ABL transformation by interacting with other proteins. Another IRF-4 binding protein is IBP (31). In addition to inhibiting IRF-4’s DNA binding, IBP has been shown to function as an activator for Rac (38, 39). It has been shown that Rac1 and Rac2 are key regulators of leukemogenesis induced by BCR/ABL (40). Thus, IRF-4 interaction with IBP may play a role in suppressing BCR/ABL transformation. This possibility will be tested in the future.

Our result showed that IRF-4 is a more potent inhibitor of BCR/ABL transformation than IRF-8 (Fig. 2). This seems puzzling at first, since IRF-4 KO mice, unlike IRF-8 KO mice, do not develop a myeloproliferative disease. A likely explanation, however, is the differential expression levels of IRF-4 and IRF-8 in myeloid cells. It has been shown that IRF-8 is more active than IRF-4 in myeloid cells due to its higher abundance (41). So even though IRF-4 is a more potent tumor suppressor in myeloid cells, its low expression in these cells makes it a weak tumor suppressor overall. However, our finding suggests that therapies up-regulating IRF-4 may be effective in treating CML.

IRF-4 has diverse functions in different cell contexts. It has been shown that IRF-4, as well as IRF-8, regulates Ikaros and Aiolos expression (18). These two genes belong to the Ikaros family of zinc-finger transcription factors and inhibit the expression of the surrogate light chain and promote cell cycle withdrawal in pre-B-cell development. IRF-4 also regulates the
gene encoding the chemokine receptor Cxcr4 (42). The up-regulation of Cxcr4, the receptor for CCL12, can promote migration of pre-B cells away from IL-7-expressing stroma cells, an event that is necessary for pre-B cell development. These studies show that IRF-4 and IRF-8 regulate genes important for the transition from large, cycling pre-B to small, resting pre-B cells. The transcriptional regulatory functions of IRF-4/8 may underlie, at least in part, the mechanism by which IRF-4/8 function as tumor suppressors in early B-cell development. IRF-4, therefore, may exert tumor suppressor function through different mechanisms in different cell contexts. The mechanisms by which IRF-4 functions as a tumor suppressor in other cell contexts will be studied in the future.

Acknowledgments—We thank Dr. Harinder Singh of the University of Chicago for providing the DNA binding and PPL 1 binding mutants of IRF-4, Dr. Douglas Golenbock of the University of Massachusetts Medical School for providing the MyD88 KO mice, and Craig Strop for editing the manuscript.

REFERENCES

1. Marecki, S., and Fenton, M. J. (2002) The role of IRF-4 in transcriptional regulation. J. Interferon Cytokine Res. 22, 121–133
2. Shaffer, A. L., Emre, N. C., Romesser, P. B., and Staudt, L. M. (2009) IRF4: Immunity. Malignancy? Therapy? Clin. Cancer Res. 15, 2954–2961
3. Iida, S., Rao, P. H., Butler, M., Corradini, P., Boccardo, M., Klein, B., Chaganti, R. S., and Dalla-Favera, R. (1997) Deregression of MUM1/IRF4 by chromosomal translocation in multiple myeloma. Nat. Genet. 17, 226–230
4. Ito, M., Iida, S., Inagaki, H., Tsuboi, K., Komatsu, H., Yamaguchi, M., Nakamura, N., Suzuki, R., Seto, M., Nakamura, S., Morishima, Y., and Ueda, R. (2002) MUM1/IRF4 expression is an unfavorable prognostic factor in B-cell chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL). Jpn. J. Cancer Res. 93, 685–694
5. Shaffer, A. L., Emre, N. C., Lamy, L., Ngo, V. N., Wright, G., Xiao, W., Powell, J., Dave, S., Yu, X., Zhao, H., Zeng, Y., Chen, B., Epstein, J., and Staudt, L. M. (2008) IRF4 addiction in multiple myeloma. Nature 454, 226–231
6. Honma, K., Udono, H., Kohno, T., Yamamoto, K., Ogawa, A., Takemori, T., Kumatoki, A., Suzuki, S., Matsuyama, T., and Yui, K. (2005) Interferon regulatory factor 4 negatively regulates the production of proinflammatory cytokines by macrophages in response to LPS. Proc. Natl. Acad. Sci. U.S.A. 102, 16001–16006
7. Negishi, H., Obba, Y., Yanai, H., Takaoka, A., Honma, K., Yui, K., Matsuyama, T., Taniguchi, T., and Honda, K. (2005) Negative regulation of Toll-like-receptor signaling by IRF4. Proc. Natl. Acad. Sci. U.S.A. 102, 15989–15994
8. Taniguchi, T., Ogawara, K., Takaoka, A., and Tanaka, N. (2001) IRF family of transcription factors as regulators of host defense. Annu. Rev. Immunol. 19, 623–655
9. Chiron, D., Bekeredjian-Ding, I., Pellat-Deceunynck, C., Bataille, R., and Jego, G. (2008) Toll-like receptors: lessons to learn from normal and malignant human B cells. Blood 112, 2205–2213
10. Huang, B., Zhao, J., Unkeless, J. C., Feng, Z. H., and Xiong, H. (2008) TLR signaling by tumor and immune cells: a double-edged sword. Oncogene 27, 218–224
11. Rakoff-Nahoum, S., and Medzhitov, R. (2009) Toll-like receptors and cancer. Nat. Rev. Cancer 9, 57–63
12. Takaoka, A., Yanai, H., Kondo, S., Duncan, G., Negishi, H., Mizutani, T., Kano, S., Honda, K., Obba, Y., Mak, T. W., and Taniguchi, T. (2005) Integral role of IRF-5 in the gene induction programme activated by Toll-like receptors. Nature 434, 243–249
13. Tamura, T., and Ozato, K. (2002) ICSBP/IRF-8: its regulatory roles in the development of myeloid cells. J. Interferon Cytokine Res. 22, 145–152
14. Holtzschke, T., Löhler, J., Kanno, Y., Fehr, T., Giese, N., Rosenbauer, F., Lou, J., Knobeloch, K. P., Gabriele, L., Waring, J. F., Bachmann, M. F., Zinkernagel, R. M., Morse, H. C., 3rd, Ozato, K., and Horak, I. (1996) Immunodeficiency and chronic myelogenous leukemia-like syndrome in mice with a targeted mutation of the ICSBP gene. Cell 87, 307–317
15. Ren, R. (2005) Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukemia. Nat. Rev. Cancer 5, 172–183
16. Hao, S. X., and Ren, R. (2000) Expression of interferon consensus sequence binding protein (ICSBP) is downregulated in Bcr-Ab1-induced murine chronic myelogenous leukemia-like disease, and forced coexpression of ICSBP inhibits Bcr-Ab1-induced myeloproliferative disorder. Mol. Cell. Biol. 20, 1149–1161
17. Lu, R., Medina, K. L., Lankci, D. W., and Singh, H. (2003) IRF-4 orchestrates the pre-B-to-B transition in lymphocyte development. Genes Dev. 17, 1703–1708
18. Ma, S., Pathak, S., Trinh, L., and Lu, R. (2008) Interferon regulatory factors 4 and 8 induce the expression of Ikaros and Aiolos to down-regulate pre-B-cell receptor and promote cell-cycle withdrawal in pre-B-cell development. Blood 111, 1396–1403
19. Lu, R. (2008) Interferon regulatory factor 4 and 8 in B-cell development. Trends Immunol. 29, 487–492
20. Ortmann, C. A., Burchart, A., Höökse, K., Nitsche, A., Wittig, B., Neubauer, A., and Schmidt, M. (2005) Down-regulation of interferon regulatory factor 4 gene expression in leukemic cells due to hypermethylation of CpG motifs in the promoter region. Nucleic Acids Res. 33, 6895–6905
21. Schmidt, M., Hochhaus, A., König-Merediz, S. A., Brendel, C., Proba, J., Hoppe, G. J., Wittig, B., Ehninger, G., Hehlmann, R., and Neubauer, A. (2000) Expression of interferon regulatory factor 4 in chronic myeloid leukemia: correlation with response to interferon alpha therapy. J. Clin. Oncol 18, 3331–3338
22. Klein, F., Feldhahn, N., Mooster, J. L., Sprangers, M., Hofmann, W. K., Wernet, P., Wartenberg, M., and Müschen, M. (2005) Tracing the pre-B to immature B cell transition in human leukemia cells reveals a coordinated sequence of primary and secondary IGK gene rearrangement, IGK deletion, and IGL gene rearrangement. J. Immunol. 174, 367–375
23. Schmidt, M., Hochhaus, A., Nitsche, A., Hehlmann, R., and Neubauer, A. (2001) Expression of nuclear transcription factor interferon consensus sequence binding protein in chronic myeloid leukemia correlates with pretreatment risk features and cytogenetic response to interferon-alpha. Blood 97, 3648–3650
24. Jo, S. H., Schatz, J. H., Acquaviva, J., Singh, H., and Ren, R. (2010) Cooperation between deficiencies of IRF-4 and IRF-8 promotes both myeloid and lymphoid tumorigenesis. Blood 116, 2759–2767
25. Acquaviva, J., Chen, X., and Ren, R. (2008) IRF-4 functions as a tumor suppressor in early B-cell development. Blood 112, 3798–3806
26. Coligan, J. E., Margulies, D. H., Shevach, E. M., and Strober, W. (1996) Current Protocols in Immunology, New York
27. Brass, A. L., Zhu, A. Q., and Singh, H. (1999) Assembly requirements of PU.1-Pip (IRF-4) activator complexes: inhibiting function in vivo using fused dimers. EMBO J. 18, 977–991
28. Pear, W. S., Nolan, G. P., Scott, M. L., and Baltimore, D. (1993) Production of high-titer helper-free retroviruses by transient transfection. Proc. Natl. Acad. Sci. U.S.A. 90, 8392–8396
29. Gross, A. W., Zhang, X., and Ren, R. (1999) Bcr-Ab1 with an SH3 deletion retains the ability to induce a myeloproliferative disease in mice, yet c-Ab1 activated by an SH3 deletion induces only lymphoid malignancy. Mol. Cell. Biol. 19, 6918–6928
30. Sciammas, R., Shaffer, A. L., Schatz, J. H., Zhao, H., Staudt, L. M., and Singh, H. (2006) Graded expression of interferon regulatory factor-4 coordinates isotype switching with plasma cell differentiation. Immunity 25, 225–236
31. Chen, Q., Yang, W., Gupta, S., Biswas, P., Smith, P., Bhagat, G., and Pernis, A. B. (2008) IRF-4-binding protein inhibits interleukin-17 and interleukin-21 production by controlling the activity of IRF-4 transcription factor. Immunity 29, 899–911
32. Mamane, Y., Sharma, S., Petropoulos, L., Lin, R., and Hiscott, J. (2000) Posttranslational regulation of IRF-4 activity by the immunophilin FKBP52. Immunity 12, 129–140

JANUARY 13, 2012 • VOLUME 287 • NUMBER 3 JOURNAL OF BIOLOGICAL CHEMISTRY 1777
33. Himmelmann, A., Riva, A., Wilson, G. L., Lucas, B. P., Thevenin, C., and Kehrl, J. H. (1997) PU.1/Pip and basic helix loop helix zipper transcription factors interact with binding sites in the CD20 promoter to help confer lineage- and stage-specific expression of CD20 in B lymphocytes. *Blood* **90**, 3984–3995

34. Marecki, S., Riendeau, C. J., Liang, M. D., and Fenton, M. J. (2001) PU.1 and multiple IFN regulatory factor proteins synergize to mediate transcriptional activation of the human IL-1 beta gene. *J. Immunol.* **166**, 6829–6838

35. Brass, A. L., Kehrli, E., Eisenbeis, C. F., Storb, U., and Singh, H. (1996) Pip, a lymphoid-restricted IRF, contains a regulatory domain that is important for autoinhibition and ternary complex formation with the Ets factor PU.1. *Genes Dev.* **10**, 2335–2347

36. Rakoff-Nahoum, S., and Medzhitov, R. (2007) Regulation of spontaneous intestinal tumorigenesis through the adaptor protein MyD88. *Science* **317**, 124–127

37. Lee, S. H., Hu, L. L., Gonzalez-Navajas, J., Seo, G. S., Shen, C., Brick, J., Herdman, S., Varki, N., Corr, M., Lee, J., and Raz, E. (2010) ERK activation drives intestinal tumorigenesis in Apc(min/+) mice. *Nat. Med.* **16**, 665–670

38. Gupta, S., Fanzo, J. C., Hu, C., Cox, D., Jang, S. Y., Lee, A. E., Greenberg, S., and Pernis, A. B. (2003) T cell receptor engagement leads to the recruitment of IBP, a novel guanine nucleotide exchange factor, to the immunological synapse. *J. Biol. Chem.* **278**, 43541–43549

39. Gupta, S., Lee, A., Hu, C., Fanzo, J., Goldberg, L., Cattoretti, G., and Pernis, A. B. (2003) Molecular cloning of IBP, a SWAP-70 homologous GEF, which is highly expressed in the immune system. *Human Immunol.* **64**, 389–401

40. Thomas, E. K., Cancelas, J. A., Zheng, Y., and Williams, D. A. (2008) Rac GTPases as key regulators of p210-BCR-ABL-dependent leukemogenesis. *Leukemia* **22**, 898–904

41. Kanno, Y., Levi, B. Z., Tamura, T., and Ozato, K. (2005) Immune cell-specific amplification of interferon signaling by the IRF-4/8-PU.1 complex. *J. Interferon Cytokine Res.* **25**, 770–779

42. Johnson, K., Hashimshony, T., Sawai, C. M., Pongubala, J. M., Skok, J. A., Aifantis, I., and Singh, H. (2008) Regulation of immunoglobulin light-chain recombination by the transcription factor IRF-4 and the attenuation of interleukin-7 signaling. *Immunity* **28**, 335–345