IRF4: Immunity. Malignancy! Therapy?

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Abstract

IRF4, a member of the Interferon Regulatory Factor (IRF) family of transcription factors, is expressed in cells of the immune system, where it transduces signals from various receptors to activate or repress gene expression. IRF4 expression is a key regulator of several steps in lymphoid-, myeloid-, and dendritic-cell differentiation, including the differentiation of mature B cells into antibody-secreting plasma cells. IRF4 expression is also associated with many lymphoid malignancies, with recent evidence pointing to an essential role in multiple myeloma, a malignancy of plasma cells. Interference with IRF4 expression is lethal to multiple myeloma cells, irrespective of their genetic etiology, making IRF4 an ‘Achilles’ heel’ that may be exploited therapeutically.

Background

The Interferon Regulatory Factor (IRF) family comprises a broadly expressed set of transcription factors first described as downstream regulators of interferon signaling. Some IRFs also participate in signal transduction through pattern-recognition receptors, such as the Toll-like receptors (TLRs). IRF4 (also known as pip, MUM1, LSIRF, NFEM5, and ICSAT) is an IRF-family member that is restricted in expression to the immune system, a property it shares with its closely related family member IRF8. IRF4 and IRF8 have evolved as critical mediators of lymphoid-, myeloid-, and dendritic-cell development (1–5).

Unlike other IRF-family members, IRF4 is not induced by interferon, but rather by diverse mitogenic stimuli, including antigen receptor engagement, lipopolysaccharides, and CD40 signaling (6–8). These stimuli all activate the NF-κB pathway, which leads to IRF4 promoter activation by NF-κB heterodimers (7–10). In addition, IRF4 transcription can be activated by the cytokine IL-4, implicating the transcription factor STAT6 which leads to IRF4 dimers (7–10). In addition, IRF4 transcription can be activated by the cytokine IL-4, implicating the transcription factor STAT6 (7–10). In addition, IRF4 transcription can be activated by the cytokine IL-4, implicating the transcription factor STAT6, which leads to IRF4 dimers (7–10).

By contrast, IRF8 lacks a transactivation domain; therefore, its expression is repressed by the inhibitory domain (15). However, IRF4 can bind with high avidity to the 3’ enhancer of both κ and λ immunoglobulin light chains in conjunction with the ETS-family transcription factor PU.1 or the closely related factor SPIB (17–19). Cooperative DNA binding is facilitated by two separate protein-protein interfaces between IRF4 and PU.1; a strong interaction is mediated by a C-terminal regulatory domain of IRF4 and PU.1’s phosphorylated PEST domain. In the absence of PU.1, this IRF4 regulatory domain inhibits DNA binding, but the interaction with PU.1 relieves this autoinhibition (20, 21).

Alone, IRF4 binds DNA weakly due to its C-terminal autoinhibitory domain (15). However, IRF4 can bind with high avidity to the 3’ enhancers of both κ and λ immunoglobulin light chains in conjunction with the ETS-family transcription factor PL1 or the closely related factor SPIB (17–19). Cooperative DNA binding is facilitated by two separate protein-protein interfaces between IRF4 and PU.1; a strong interaction is mediated by a C-terminal regulatory domain of IRF4 and PU.1’s phosphorylated PEST domain. In the absence of PU.1, this IRF4 regulatory domain inhibits DNA binding, but the interaction with PU.1 relieves this autoinhibition (20, 21). A second protein-protein interface exists between the DNA-binding domains of IRF4 and PU.1 (15, 22). When binding with a partner such as PL1.1 or SPIB, IRF4 regulates gene expression by binding a composite DNA element called ETS/ISRE-consensus element (EICE), which has the consensus sequence 5’-GGAANNAGA-3’ that fuses the ETS-binding motif (5’-GGAA-3’) with the IRF4-binding motif (5’-AANNGAAA-3’) (refs. 2, 15, 16).

Although IRF4 can weakly interact with this element alone, cooperative binding with PL1.1 increases its avidity by ~5-fold (22). IRF8 can similarly interact with PL1.1, by virtue of its structural similarity with IRF4, but other IRF-family members cannot (22, 23). Composite EICE elements also mediate cooperative binding of IRF4/IRF8 and PU.1/SPIB on other genes, including TLR4 and CIITA (24, 25).

Additional protein-protein interactions between IRF4 and other regulatory factors modulate its DNA-binding properties and/or transactivation potential. The EICE site in the Ig 3’ κ light-chain enhancer is adjacent to a binding site for the E-box...
protein E47, encoded by the E2A gene (26). Protein-protein interactions between IRF4 and E47 increase the ability of these factors to activate transcription through this element by 50- to 100-fold (26). Similar transcriptional synergy between IRF4 and E47 operates at the CIITA and CD20 loci (25, 27). In transient transfection studies, IRF4 can also interact and cooperate with STAT6 to induce the STAT6-responsive gene CD23, an effect that can be blocked by physical interaction between IRF4 and either BCL6 or PRDM1 (7, 28). IRF4 can also functionally cooperate with the transcription factor NFATC2 to synergistically regulate the IL4 promoter in T cells (29). Finally, the ability of IRF4 to bind to the promoters of the proinflammatory cytokines IL-17 and IL-21 can be blocked by binding of IRF4 to a small GTPase termed IBP (30, 31). Interestingly, mice deficient in IBP develop a virulent autoimmune disease involving excessive IL-17 and IL-21 production, but breeding these mice to an IRF4 knockout strain ameliorates this disease (30).

IRF4 is an essential regulator at multiple steps in B-cell differentiation. Both IRF4 and IRF8 are required in a redundant fashion to regulate the pre-B-cell transition (32). B cells deficient in both IRF4 and IRF8 are arrested at the large pre-B-cell stage in which cells have undergone VDJ recombination at the immunoglobulin heavy-chain locus, express cytoplasmic immunoglobulin heavy chain, have elevated expression of the pre-B-cell receptor (preBCR) on the cell surface, and are rapidly proliferating. Mechanistically, IRF4 or IRF8 downregulate expression of preBCR components by inducing the Ikaros and Aiolos transcription factors, thereby terminating preBCR-driven proliferation (33). In small pre-B cells, IRF4 activates immunoglobulin light-chain gene rearrangement by directly binding to the \( \lambda \) and \( \kappa \) immunoglobulin enhancers (34). Additionally, IRF4 upregulates CXCR4, the receptor for the chemokine SDF-1. The potential consequence is the migration of small pre-B cells toward bone-marrow stromal cells that express SDF-1, providing a mechanism for B-cell differentiation in vivo (35). Such migration could further augment immunoglobulin light-chain gene rearrangement since IL-7 inhibits this process (34). The ability of IRF4 to promote immunoglobulin light-chain rearrangement serves as an additional important function in central B-cell tolerance by "editing" self-reactive B-cell receptors (36). Exposure of a self-reactive B cell to its cognate self-antigen upregulates IRF4, promoting immunoglobulin light-chain rearrangement and replacement, which enables some B-cell progeny to escape deletion and populate the peripheral lymphoid organs.

IRF4 plays a critical and nonredundant role in the adaptive immune responses of mature B cells (Fig. 1) (ref. 6). Without IRF4, mature B cells accumulate in increased numbers in the spleen and lymph node, although there is a quantitative defect in the percentage of IgM⁺, IgD⁻, CD23⁻ mature B cells. Most striking is the complete absence of GC formation in response to antigenic challenge. Consequently, IRF4-deficient mice do not produce antigen-specific antibody upon immunization. The failure of IRF4-deficient cells to undergo GC differentiation may be due to a B-cell intrinsic deficit since IRF4-deficient B cells proliferate poorly upon BCR crosslinking or LPS treatment in vitro, although the proliferative response to anti-CD40 stimulation remains intact (6). Cell division in response to LPS is initially normal in IRF4-deficient B cells, but they do not progress through later rounds of division, apparently due to increased cell death (13). The inability of IRF4-deficient B cells to proliferate and survive upon B-cell receptor stimulation may be sufficient to prevent GC B-cell differentiation, but it is also conceivable that IRF4 may regulate a key gene(s) required to promote GC differentiation.

Serum immunoglobulins of all isotypes are exceedingly low in IRF4-deficient mice (6). This deficit may be attributed in part to the lack of GC formation in these animals, but another contributing factor is a severe impairment in antibody secretion (13, 37). In vitro, neither LPS nor anti-CD40 treatment induces IRF4-deficient B cells to secrete antibody. This failure can be traced to a defect in the generation of antibody-secreting B cells and to a decrease in the amount of immunoglobulin secretion on a per cell basis (13, 37). In this regard, it is interesting that a quantitative trait locus in inbred mice for serum IgM levels maps precisely to the IRF4 locus (38).

IRF4 is also required for immunoglobulin class switch recombination (CSR) (refs. 13, 37). IRF4-deficient B cells fail to upregulate AID, the critical enzyme that mediates CSR (13, 37). Ectopic provision of AID to IRF4-deficient cells improved CSR, but ectopic restoration of IRF4 was more effective (13), suggesting that IRF4 may control CSR in other ways. CSR requires sterile transcription through the immunoglobulin switch regions (39). Although sterile Cy1 transcription was activated normally in IRF4-deficient B cells (37), it is possible that a relative defect in sterile Cy1 transcription may impair CSR in these cells (40).

IRF4-deficient mice completely lack immunoglobulin-secreting plasma cells (6). Three lines of evidence demonstrate that this phenotype reflects a B-cell-intrinsic requirement for IRF4 during terminal differentiation of B cells to plasma cells. First, purified IRF4-deficient B cells are incapable of forming plasma cells under a variety of differentiation conditions in vitro (13, 37). Second, conditional ablation of the IRF4 locus in B cells blocks plasmacytic differentiation in vivo (37). Third, ectopic expression of IRF4 promotes plasmacytic differentiation (13). Although IRF4 is expressed at varying levels throughout B-cell development, its expression peaks in plasma cells (15). Most GC B cells lack IRF4 expression and instead express IRF8 (41, 42). The few GC B cells that do express IRF4 have lost expression of BCL6 and Ki67 and express the master regulator of plasma-cell differentiation, PRDM1. These characteristics suggest that these IRF4⁺ GC cells are preparing to leave the GC and differentiate into plasma cells (43). Upon ectopic expression of IRF4 in mature B cells in vitro, only those cells with the highest IRF4 expression differentiate into plasma cells, suggesting a model in which graded expression of IRF4 during B-cell activation and plasmacytic differentiation modulates its biological effect (Fig. 1) (ref. 13).

Outside the B-cell lineage, IRF4 is essential for several stages of T-cell and myeloid-cell differentiation. IRF4 is emerging as a critical regulator of T-helper-cell differentiation, playing a required role in both Th2 and Th17 development by controlling cytokine expression and apoptosis (29, 44–47). Strikingly, the defective Th17 differentiation in IRF4-deficient animals renders them insensitive to experimental autoimmune encephalomyelitis (45). In addition, both IRF4 and IRF8 control the differentiation of various dendritic-cell populations. IRF4 expression is solely required for CD4-positive dendritic-cell development, whereas IRF8 is solely required for CD8a-positive dendritic-cell differentiation. Both factors function in a redundant fashion to promote CD4, CD8-negative dendritic-cell and plasmacytoid
dendritic-cell differentiation (48). IRF4 expression in myeloid lineage interferes with TLR signaling by competing with Irf5 for binding to the Myd88 protein, the key signaling adaptor that transmits signals from these receptors (49). Accordingly, IRF4-deficient mice are hypersensitive to CpG-induced shock, which is mediated by TLR9 signaling.

Finally, it is important to emphasize that IRF4-deficient mice have no apparent phenotypes outside of the lymphoid and myeloid lineages, in keeping with the restricted expression of IRF4 in these cell types. Therefore, potential therapies aimed at IRF4 would have restricted and potentially manageable off-target toxicities within the hematopoietic system (see below).

**Clinical-Translational Advances**

A recurring theme in the pathogenesis of B-cell malignancies is the ability of transcription factors to serve as oncoproteins when they are deregulated by chromosomal translocation, amplification, or mutation. Indeed, chromosomal abnormalities involving the transcription factors MYC and BCL6 are among the most common oncogenic events in multiple myeloma (MM) and diffuse large B-cell lymphoma, respectively. In rare cases of MM, the IRF4 gene is brought under the control of immunoglobulin heavy-chain regulatory regions by a chromosomal translocation, providing genetic evidence that IRF4 can function as an oncogene (50, 51). Recently, recurrent translocations involving the IRF4 locus were also identified in T-cell lymphomas, including peripheral T-cell lymphoma (PTCL), unspecified (6% of cases), and cutaneous anaplastic large-cell lymphoma (57% of cases) (ref. 52). Although the exact nature of these translocation breakpoints was not determined, these observations may indicate an important role for IRF4 in the pathogenesis of certain T-cell lymphomas. Although IRF4 translocations establish its oncogenic potential, cancer cells can be dependent upon IRF4 even if the IRF4 locus is not genetically altered (see below).

The expression of IRF4 in human malignancies mirrors its expression in lymphoid activation and differentiation. Two oncogenic viruses, HTLV-I and EBV, activate the NF-κB pathway and consequently elevate IRF4 expression. HTLV-I, the etiologic agent in adult T-cell leukemia (ATL), encodes an NF-κB transactivator, tax, which upregulates IRF4 (53). In some ATL cases, the T-cell cytokine IL-15 has been implicated as a growth factor (54). Since IRF4 can transactivate the gene encoding the IL-15 receptor α chain, it is possible that IRF4 contributes to a growth-promoting autocrine loop in these cases (55). The EBV-encoded protein LMP1 activates the NF-κB pathway, thereby upregulating IRF4 (56). EBV infection transforms mature human B cells into lymphoblastoid cell lines. Knockdown of IRF4 in EBV+ lymphoblastoid cells decreases cell division and increases apoptosis. EBV has also been implicated in many lymphomas, including primary central nervous system lymphoma, Hodgkin’s lymphoma, and rare nodal diffuse large B-cell lymphomas (DLBCLs). In primary central nervous system lymphoma, expression of IRF4 correlates with expression of LMP1 (56).

Among malignancies of mature B cells, IRF4 is characteristic at high levels in the activated B-cell-like (57) subtype of DLBCL, which is the least curable subtype by current therapy (58, 59). DLBCL subtypes differ from one another by the expression of thousands of genes owing to their derivation from distinct stages of B-cell differentiation (60). The ABC DLBCL subtype appears to be generated from post-GC B cells that are blocked during the process of plasmacytic differentiation. A hallmark of the ABC DLBCL subtype is constitutive activation of the NF-κB pathway, a process mediated by the signaling adapter CARD11 (61, 62). IRF4 expression is driven by the constitutive NF-κB activity in this DLBCL subtype (63). By contrast, IRF4 is expressed at low levels in the GC B-cell-like (GCB) DLBCL subtype, which is derived from normal GC B cells that have conspicuously low activity of the NF-κB pathway (12, 64).

Although the functional role of IRF4 in ABC DLBCL remains to be fully elucidated, it is intriguing that the IRF4-interacting factor SPIB has emerged as an oncogene in this lymphoma subtype (65, 66). SPIB is deregulated by recurrent amplifications in roughly one-quarter of ABC DLBCL cases and by a translocation in an ABC DLBCL cell line, but these events occur only rarely in GCB DLBCLs. Moreover, SPIB knockdown was lethal to ABC DLBCL cell lines, but not to GCB DLBCL cell lines. These results raise the intriguing possibility that IRF4 may cooperate with SPIB to promote the malignant phenotype of ABC DLBCL. However, the role of IRF4 in ABC DLBCL is complicated by the fact that it can promote plasmacytic differentiation of normal B cells by binding and transactivating the gene encoding PRDM1 (Fig. 1) (ref. 13). Interestingly, many ABC DLBCLs have inactivated PRDM1, either by deletion, translocation, or mutation, thereby rendering it insensitive to the action of IRF4 (67, 68). Another way to prevent plasmacytic differentiation is to increase expression of BCCL, a direct repressor of PRDM1 (14, 69, 70). IRF4 binds to a region in BCCL’s first intron and represses its expression (10). Consequently, some DLBCLs accumulate somatic mutations in the IRF4-binding region of BCCL, thereby causing high expression of BCCL to be maintained in the face of IRF4 expression. Thus, ABC DLBCLs sustain a variety of genetic lesions that may allow for IRF4 action as an oncogene while restraining its ability to promote plasmacytic differentiation.

In chronic lymphocytic leukemia (CLL), there is a solid yet enigmatic association between polymorphisms in the IRF4 locus and the development of the disease. In a genome-wide association study, single nucleotide polymorphisms (SNPs) in the 3′ UTR region of IRF4 exhibited the strongest statistical association with CLL susceptibility, and this association was observed in several independent patient cohorts (71). The functional effect of the IRF4 alleles on IRF4 expression or function is unknown. Interestingly, the IRF4 SNP was associated more strongly with the subtype of CLL that has mutated immunoglobulin genes, presumably indicating an origin from post-GC memory B cells. Given the important role of IRF4 in plasmacytic differentiation, it is conceivable that the IRF4 SNPs influence CLL risk by impairing the differentiation of memory B cells into plasma cells. Alternatively, given the requirement for IRF4 in B-cell activation (6), the IRF4 SNPs may control an aspect of the B-cell activation program that contributes to aberrant B-cell receptor signaling in CLL.

The role of IRF4 in promoting malignancy has been most clearly shown in MM, a malignancy of plasma cells. MM presents at a peak age of 65 to 70 and accounts for ~15% of all hematological malignancies, second only to non-Hodgkin’s lymphoma. Despite vigorous clinical research efforts directed at discovering curative therapies, MM patients still have a median
survival time of just 3 years. Current therapy includes the use of alkylating agents, glucocorticoids, proteosome inhibitors, farnesylation inhibitors, and thalidomide (and its analogs) (ref. 72). An effective therapy for patients under 65 years of age combines high-dose cytotoxic agents with autologous bone-marrow transplantation to reconstitute the patient’s hematologic system (73). Treatment regimens are further limited in older patients who cannot tolerate intensive therapy, and a variety of single-drug and drug-cocktail regimens are employed in this population. Recently, the combination of dexamethasone and the thalidomide analog lenolidomide appears to be promising in relapsed and refractory MM, but the responses still do not appear to be curative (74).

The developmental and molecular biology of MM contribute to this difficulty in treatment. MM cells, like their normal plasma-cell counterparts, reside in the bone marrow, which provides a microenvironment that sustains their proliferation and survival and protects them from the cytotoxic effect of therapy (75). In addition, MM is initiated and maintained by a wide spectrum of genetic anomalies, including translocations of oncogenes to the immunoglobulin loci (p-type cyclins, c-Maf, MafB, c-myc, MMSET, FGFR3), as well as mutations of oncogenes (N-ras, K-ras) and tumor suppressors (p53, p18) (ref. 76). In addition, translocations between the immunoglobulin heavy-chain locus and the IRF4 locus have been characterized in a few MM cell lines and rare MM patient

**Fig. 1.** IRF4 regulatory networks in B-cell differentiation and malignancy. IRF4 lies at the center of regulatory networks that function at several stages of B-cell differentiation and drive malignancy. A, in resting mature B cells, IRF4 levels are low or absent due to MITF repression of the IRF4 gene. B, IRF4 expression is induced by BCR, CD40, and cytokine stimulation via NF-κB and STAT factors in activated B cells, and MITF repression is relieved. IRF4 acutely drives MYC and PRDM1 expression and utilizes a feedback mechanism to drive its own expression. This results in a burst of cell division (driven by MYC) and subsequent differentiation of a proportion of the cells into short-lived Ig secretors (driven by PRDM1). C, germinal center (GC) B cells express IRF8, whereas IRF4 levels are low due to a lack of NF-κB activation as well as repression of IRF4 by MITF. This allows the expression of the key GC regulator, BCL6, which, in turn, represses PRDM1, the plasma-cell master regulator, locking the cells into the GC phenotype. Lack of IRF4 also contributes to the absence of MYC expression in GC B cells. D, as B cells exit the GC, they may differentiate to plasma cells. IRF4 expression, initiated by activation stimuli, becomes activation independent via IRF4 autoinduction. IRF4 represses the GC regulator BCL6 and directly induces expression of the PC regulator PRDM1. PRDM1 also directly or indirectly represses BCL6 expression, committing cells to a plasma-cell fate. In addition, PRDM1 directly represses MYC, leading to the nondividing, Ig-secreting phenotype of terminally differentiated plasma cells. E, in multiple myeloma (MM), the malignant counterpart of normal plasma cells, IRF4 represses BCL6 and promotes PRDM1 expression as in plasma cells, but both MYC and IRF4 are overexpressed. For unknown reasons, MYC is not repressed by PRDM1 in MM, leading to a positive-feedback loop between IRF4 and MYC, as seen in activated B cells. Thus, MM fuses the activated B-cell and plasma-cell IRF4-driven gene-expression programs, leading to malignant transformation and cell division. Black arrows/lines, active regulation; gray arrows/lines, inactive regulation; black letters, active factor; gray letters, inactive factor.
samples (50, 77). This genetic heterogeneity is reflected in the gene-expression patterns of MM patient samples, which have been used to classify MM into seven molecular subtypes (78).

Recently, we have designed and employed an RNA interference genetic screen to identify new therapeutic targets in cancer (61). This so-called “Achilles’ heel” screen identifies genes that are critical for tumor proliferation and/or survival. Applying this Achilles’ heel screen in MM revealed IRF4 to be an essential gene in this malignancy (14). Knockdown of IRF4 by RNA interference induces a rapid and profound non-apoptotic cell death in all MM cell lines tested, regardless of the suite of genetic anomalies inherent to each line. The genetic repertoire controlled by IRF4 was defined by using gene-expression profiling after IRF4 knockdown together with genome-wide chromatin immunoprecipitation analysis of IRF4-binding sites (14). These efforts revealed that IRF4 controls an aberrant gene-expression program in MM that fuses the gene-expression programs of activated B cells and normal plasma cells. Several key metabolic pathways lie downstream of IRF4 in MM cells, including lipid and cholesterol biosynthesis, glucose metabolism, general transcriptional regulation, and cell-cycle progression. The toxicity of IRF4 knockdown in MM appears to be “death by a thousand cuts” since numerous IRF4 target genes play crucial roles in the proliferation and survival of MM cells (Fig. 2).

A particularly noteworthy IRF4 target gene in MM is MYC (Figs. 1 and 2) (ref. 14). IRF4 binds directly to the MYC promoter region in MM cells and transactivates its expression. Conversely, MYC transactivates IRF4 by binding to an evolutionarily conserved intronic region, creating a positive autoregulatory feedback loop. The expression of MYC in the malignant cells of MM is decidedly abnormal since normal plasma cells do not express MYC due to repression by PRDM1 (Fig. 1). MYC is frequently deregulated by translocation or amplification in MM (79), events that presumably upregulate both MYC and IRF4. Indeed, the expression of IRF4 and MYC is positively correlated in primary bone-marrow samples from patients with MM (14). It is important to emphasize that most MMs do not have genetic lesions in the IRF4 locus but are nonetheless completely dependent upon the aberrant genetic program controlled by IRF4. IRF4 dependency in MM is thus a prime example of a newly appreciated phenomenon termed “non-oncogene addiction” in which cancer cells develop an exaggerated reliance upon a normal cellular protein as a result of their genetic and biological abnormalities (80).

Although transcription factors have been considered to be intractable therapeutic targets, recent successful targeting of p53 (81) and BCL6 (82) using small-molecule inhibitors provides hope that IRF4 can be attacked as an Achilles’ heel of MM and other IRF4-dependent malignancies. There are several aspects of IRF4 biology that may be exploited for therapeutic purposes (Fig. 2). First, therapies that target the transcription of IRF4 could be envisioned. In malignancies in which IRF4 is dependent upon NF-κB signaling—ABC DLBCL, ATL, and EBV-associated processes—inhibitors of IkB kinase β would be effective. Although NF-κB is constitutively active in many cases of MM (83, 84), IRF4 expression is not dependent upon this pathway since MM cell lines without NF-κB activation nonetheless have high IRF4 expression. Rather, the expression of IRF4 in MM reflects the constitutive high expression of IRF4 that is also observed in normal plasma cells (13, 14, 37). The mechanisms responsible for IRF4 expression in normal and malignant plasma cells are largely unknown, but, interestingly, IRF4 binds to its own promoter region, creating the potential for positive autoregulation (Figs. 1 and 2). Such autoregulation might create a therapeutic opportunity since any maneuver that would downregulate IRF4 expression or activity would secondarily downregulate IRF4 transcription, thereby potentiating the
effect. In addition, MYC and IRF4 form a positive autoregulatory loop (Figs. 1 and 2), implying that any therapy targeting IRF4 transcription would have the added benefit of decreasing MYC transcription. Of interest in this regard is a recent study showing that the protein kinase C β inhibitor, enzastaurin, has antiproliferative and proapoptotic activity in MM and coordinately downregulates expression of both IRF4 and MYC (85).

A second therapeutic opportunity may be provided by the fact that IRF4 binds DNA cooperatively with several DNA-binding factors, notably the ETS-family proteins PU.1 and SPIB (Fig. 2). The IRF4-PU.1 interaction requires phosphorylation of PU.1 on a single serine in its PEST domain (17), and this serine is conserved in SPIB. Casein kinase II can phosphorylate this site in vitro, but the responsible kinase in vivo has not been identified. It is conceivable that kinase inhibitors that block PU.1 and possibly SPIB phosphorylation might be exploited for the treatment of B-cell malignancies that express these ETS factors together with IRF4, such as ABC DLBCL. Since PU.1 and SPIB are not expressed in MM, it is currently unclear whether IRF4 requires other cooperating transcription factors to regulate gene expression in MM and plasma cells.

A third potential strategy to inhibit IRF4 might be to alter its posttranslational modifications (Fig. 2). IRF4 appears to undergo a regulated conformational change by interacting with a member of the immunophilin family, FKBP52 (86). FKBP52 is a peptidyl-prolyl isomerase that associates with a central proline-rich domain of IRF4. FKBP52 alters IRF4 protein structure, as evidenced by altered proteolytic cleavage, and inhibits the ability of IRF4 to bind DNA and transactivate reporter constructs. Drugs that inhibit FKBP52, such as ascomycin, increase IRF4 function (86). One would predict that cellular mechanisms that release IRF4 from the inhibitory effects of FKBP52 must exist; these might provide therapeutic opportunities. A second posttranslational modification to consider is phosphorylation. The IRF4 homolog IRF8 is tyrosine phosphorylated in cells that overexpress this factor, although the responsible kinase has not been identified (87).

Finally, it is important to envision potential side effects of a therapy aimed at IRF4. The phenotypes of IRF4-deficient mice are strictly limited to the immune system, including defects in the differentiation of plasma cells and certain dendritic-cell subsets as well as in lymphocyte activation. Notably, mice lacking one allele of IRF4 are phenotypically normal (6), yet an ~50% knockdown of IRF4 mRNA and protein was sufficient to kill myeloma cell lines (14). Thus, a therapeutic window could exist in which IRF4-directed therapy would kill IRF4-addicted malignant cells while sparing normal cells.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Molecular Pathways

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