ATF4: a novel regulator of cell growth

Activating transcription factor-4 (ATF4) is a member of the ATF/CREB subfamily of basic region-leucine zipper proteins. ATF4 forms homodimers or heterodimers by interacting with itself or other proteins through its leucine zipper motif. Potential partners of ATF4 include members of the AP-1 and C/EBP families, c-maf, and Nfe2-related factors Nrf1 and Nrf2. Previous studies have shown that ATF4 is required for lens formation in mice. Masuoka and Townes (page 736) now report that ATF4 has a role in definitive hematopoietic development. ATF4-deficient embryos are severely anemic. The fetal livers from these embryos contain fewer hematopoietic progenitors, and the colonies contain fewer cells, than controls. Whereas the lens defect is due, at least in part, to p53-dependent apoptosis, no increase in apoptosis was noted in ATF4-deficient fetal livers. Thus, the fetal anemia appears to be due to a defect in erythroid proliferation. Furthermore, ATF4-deficient mice are approximately half the size of littermate controls and have delayed hair growth. Fibroblasts from ATF4-deficient embryos have a prolonged doubling time. These results suggest that ATF4 deficiency impairs cell growth in a variety of tissues.

ATF4 joins a growing list of genes, whose disruption causes transient fetal anemia. These genes, which include “flexed”, E2f4, and Stat5a/Stat5b, are not essential for erythropoiesis. Rather, they appear to be on ancillary pathways that support erythropoiesis at specific stages of development or under conditions of stress. Additional studies are needed to determine the exact role of ATF4 in hematopoietic regulation. It is interesting to note that some of the potential dimerization partners of ATF4 may also have a role in fetal hematopoiesis. Nrf1 deficiency is associated with fetal anemia, while c-maf deficiency is associated with fetal anemia and a defect in lens development. The similarity of these phenotypes to the ATF4 phenotype raises the possibility of a functionally relevant interaction between ATF4 and Nrf1 or c-maf.

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Neutrophils at the crossroads of cytokine and RA receptors

Studies on the leukemogenic process have revealed the implication of at least 2 pathways in the generation of neutrophils, one involving a membrane receptor, GM-CSFR, and the other one, nuclear receptors of the RAR family. In tissue culture, GM-CSF induces CD34+/Lin− cells to terminal myeloid differentiation. Kit ligand (or SCF) antagonizes this effect whereas retinoids enhance it. Furthermore, cells lacking RARα/RARγ fail to undergo terminal maturation when exposed to GM-CSF or IL-3 in vitro. It came therefore as a surprise that neither gm-csf nor RARα gene knock-out disrupts granulopoiesis in vivo, suggesting redundancy and/or compensatory mechanisms. The interest in GM-CSF resurfaced with the discovery that leukemic cells in juvenile chronic myeloid leukemia (JCML) are hypersensitive to GM-CSF, followed by the demonstration of a genetic interaction between gm-csf and Nf1, a gene frequently deleted in JCML. In acute promyelocytic leukemia (APL), the involvement of the RARα locus in chromosomal translocations with 5 distinct fusion partners indicates that loss of RARα function is the primary event in leukemogenesis.

Johnson and colleagues (page 746) have now provided unequivocal evidence that GM-CSF and IL-3 enhance both ligand-dependent and ligand-independent transcriptional activity of RA receptors. Moreover, RA receptor activation is exquisitely dependent on GM-CSFR and IL-3R signaling and does not occur with activated c-Kit. This induction correlates with biologic outcome in neutrophil maturation and, together with the RAR gene knock-outs, suggests a critical role for RA receptor activation in IL-3 and GM-CSF signaling. Since loss of RAR function is associated with APL, a co-linearity between RAR and GM-CSF implies that a loss of function of GM-CSF might also be associated with myeloid leukemia. Paradoxically, GM-CSF signaling is essential in the development of a myeloproliferative disease in Nf1−/− mice. It is possible that signaling pathways have different outcomes depending on the context of the cell, a hypothesis set forth by the authors to explain the differential sensitivity of myeloid leukemias to ATRA therapy. Regardless of what future experiments will tell us, Johnson and colleagues unravel here unsuspected crosstalks between 2 disparate receptor families, bringing a novel twist to our understanding of signaling pathways.

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Another piece of the puzzle

Carton and colleagues (page 754) have added to the controversy regarding the dominant mechanism of action of rituximab in vivo. They determined the effect of a FcyRIIIa-158V/F receptor polymorphism in patients treated with 4 infusions of rituximab as initial therapy for low-risk follicular NHL. Twenty percent were homozygous for valine (158V), 35% homozygous for phenylalanine (158F), and 45% heterozygous. Patients with homogenous 158V were found to have a greater response rate and a greater molecular response at 1 year compared with patients with either the homozygous 158F or the heterozygous patients with 158F/V genotype. Despite these findings, the time-to-tumor progression did not differ between the 2 groups, likely due to the small numbers of patients. FcyRIIIa-158V/V receptors have higher affinity interaction with IgG1, promoting more efficient antibody-dependent cell-mediated cytotoxicity.
(ADCC). These results are consistent with other observations that point to the importance of ADCC as a mechanism of rituximab killing. Interestingly, there was not a clear gene-dose relationship, as the patients with homozygous 158F had a similar response rate to the patients with 158V/F. Ultimately, whether this observation is actually due to FcγRIIIa genotype or to another closely linked gene remains to be settled.

But this is not the whole story, as 67% of the 158F carriers with less efficient interaction with IgG1 had remissions and patients homozygous for 158V continue to relapse. Possible factors include other Fc receptors, complement-mediated cytotoxicity, and direct effects of antibody binding. Longer follow-up, a larger series of patients, and extension of these observations to patients receiving rituximab for relapsed disease or concurrently with chemotherapy will be important. Nevertheless, these observations may lead to strategies to augment ADCC. Options include engineering a better IgG1 antibody that allows more efficient interaction with 158F carrier genotype or increasing the number of or activation of the ADCC effector cells through administration of cytokines. Ultimately, a better understanding of these mechanisms of action and resistance will provide the rationale for new approaches that will increase response rates to antibody therapy.

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Another piece of the puzzle
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