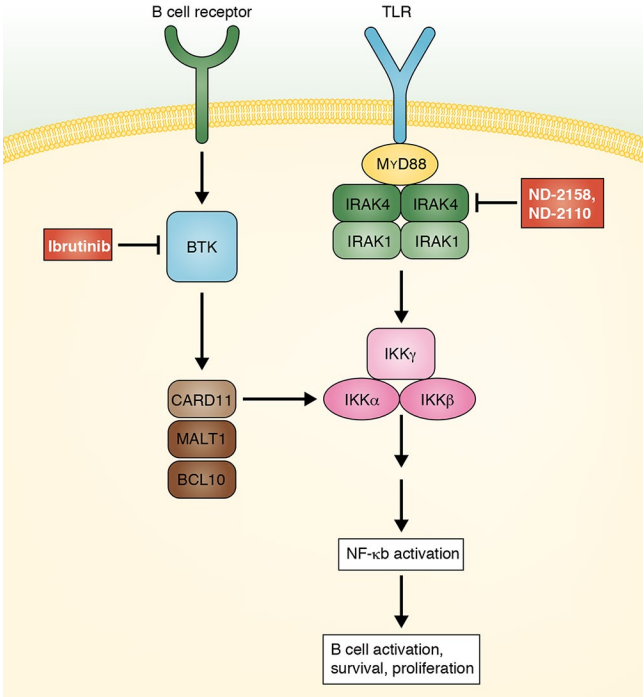
IRAK4 inhibition to shut down TLR signaling in autoimmunity and MyD88-dependent lymphomas

Insight from Ralf Küppers

An overactive Toll-like receptor (TLR) pathway is a hallmark of several autoimmune diseases and some types of B cell lymphomas. TLR signaling is a main mediator of inflammatory signals in B cells and causes NF-κB activation, which has numerous stimulatory effects, including promotion of B cell survival and proliferation. A key factor in TLR signaling is the adaptor protein MyD88. Downstream of MyD88, the interleukin-1 receptor–associated kinase 4 (IRAK4) links TLR signaling to the NF-κB pathway. Because of the overactive TLR signaling in several autoimmune diseases, and as some B cell lymphomas carry activating mutations in MyD88, there is strong interest in developing inhibitors of this pathway. MyD88 itself is not a promising target, because it is difficult to develop inhibitors for adaptor proteins. Fortunately, IRAK4 is essential for signaling through MyD88, and numerous examples of efficient kinase inhibitors exist. In this issue, Kelly et al. now succeed in identifying two novel and very promising IRAK4 inhibitors and present for the first time exciting preclinical studies with these molecules.

The two inhibitors, called ND-2158 and ND-2110, bind to the ATP pocket of IRAK4, thereby inhibiting its kinase activity. These two molecules have high affinities for IRAK4 and possess good pharmacological properties. Importantly, in a screen of 334 kinases, ND-2158 and ND-2110 proved to be highly specific for IRAK4. ND-2158 and ND-2110 were efficient in reducing disease severity in mouse models of pathological inflammatory responses. In studies with cell lines from diffuse large B cell lymphoma (DLBCL), specifically those lines of the activated B cell–like (ABC) subtype of DLBCL with a specific activating mutation in MyD88 (L265P), they showed inhibition of IRAK4 and NF-κB activity upon incubation with these inhibitors. Xenograft studies with DLBCL cell lines revealed the in vivo efficiency of ND-2158 in reducing tumor growth. As it is likely that in a potential future clinical application IRAK4 inhibition alone may not be curative, the authors studied combined application of the IRAK4 inhibitors with inhibitors of other pathogenic factors in ABC DLBCL. For example, coapplication of ibrutinib, an inhibitor of B cell receptor signaling, which is chronically active in ABC DLBCL, had synergistic effects on tumor growth in mice.



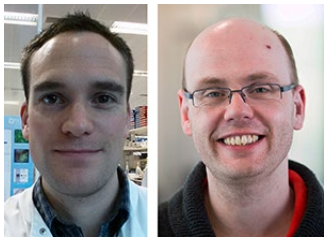
The TLR pathway is overactivated in various types of inflammatory and autoimmune diseases by ligands binding to the TLR. In 30% of ABC DLBCL and in several other B cell lymphomas, MyD88, which links TLRs to the IRAK1 and IRAK4 kinases, is affected by activating L265P mutations. Activity of IRAKs leads to activation of the canonical NF-κB pathway. IRAK4 is essential for TLR signaling through MyD88. The newly developed IRAK4 inhibitors ND-2158 and ND-2110 abrogate signaling mediated by TLR receptors or mutated MyD88. In ABC DLBCL, NF-κB is also activated though chronic active B cell receptor signaling, which involves the Bruton's tyrosine kinase (BTK). Inhibition of BTK by ibrutinib has shown clinical efficiency in first clinical studies. Notably, coapplication of ibrutininb and ND-2158 has synergistic toxic activity for ABC DLBCL cell lines in vitro and in a xenograft model. For the signaling pathways, not all components are shown.

The present study lays the ground for the development of clinical studies with the two IRAK4 inhibitors. It is impressive that this stage has already been reached, considering that MyD88 mutations were identified in ABC DLBCL only four years ago. As this type of DLBCL has the worst prognosis, and 30% of ABC DLBCL carries the L265P mutation, this new treatment approach may be valuable for a substantial fraction of DLBCL patients. Furthermore, patients affected by several other types of B cell lymphomas carrying this mutation, or patients with autoimmune diseases might also benefit from treatment with these inhibitors. Considering the recent clinical success with ibrutinib in the treatment of ABC DLBCL, it is even more promising that combined treatment with ND-2158 and ibrutinib shows synergistic therapeutic effects in these preclinical studies. What remains currently puzzling is why two ABC DLBCL lines with MyD88 mutations other than L265P were not sensitive to the IRAK4 inhibitors. This warrants further investigation.

Kelly, P.N., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20151074

Ralf Küppers, University of Duisburg-Essen, Essen: ralf.kueppers@uk-essen.de

Colon contradictions: NF-κB signaling in intestinal tumorigenesis



Insight from Robert P. Fordham (left) and Owen J. Sansom (right)

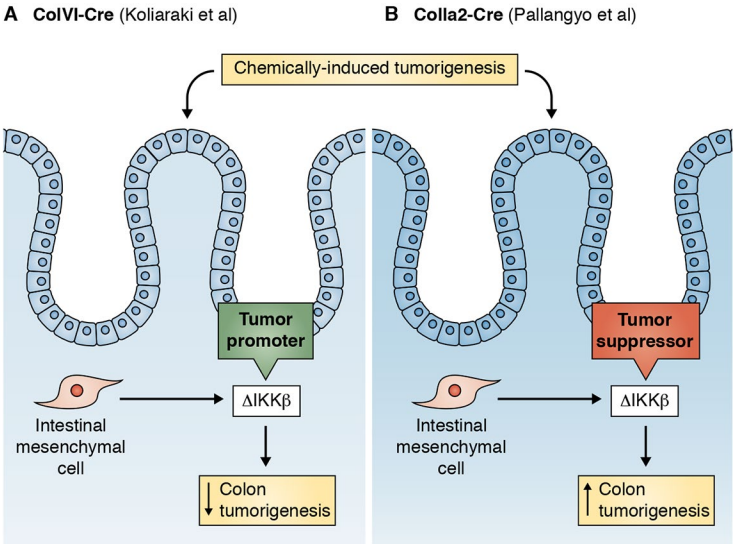
Tumor development involves a complex interplay between cancer cells and the surrounding microenvironment, including cancer-associated fibroblasts (CAFs). Whether such CAFs are tumor suppressive or tumor promoting remains a contentious issue, with obvious therapeutic implications regarding targeting of stromal cell populations in vivo.

In colorectal cancer, inflammation is strongly implicated in tumorigenesis. The NF-κB signaling pathway is of particular interest, as a key regulator of both inflammation and cancer. Previous studies from the group of Florian Greten and others have assessed the function of NF-κB signaling in intestinal epithelial cells at different stages during tumor growth. Two new studies, in this issue, have now investigated the stromal contribution of NF-κB signaling to colorectal tumorigenesis through deletion of the same gene in mesenchymal cells—with diametrically opposite results. Both groups took a broadly similar approach by deleting IKKβ (a mediator of NF-κB) in intestinal fibroblasts/CAFs, alongside chemical carcinogenesis (AOM/DSS). Using a ColVICre driver, Koliaraki et al. report that deletion of IKKβ in intestinal mesenchymal cells leads to decreased tumor incidence. Conversely, Pallangyo et al. report that deletion of IKKβ using a Col1a2Cre-ER driver stimulates intestinal epithelial cell proliferation and increased tumor size, suggesting an unexpected tumor-suppressive function for NF-κB signaling in CAFs. Interestingly, in the absence of inflammation, both models show no overt phenotype at homeostasis, suggesting that tissue damage is required.

This work can be put in the context of growing literature where the inhibition of stromal elements is hard to predict. For example, in pancreatic ductal adenocarcinoma (PDAC), activated stellate cells alter the tumor microenvironment, making the tumor refractory to drug treatment. It follows that depletion of the stroma should lead to therapeutic improvement. However, through targeted functional ablation of myofibroblasts, Raghu Kalluri and colleagues have revealed a tumor-suppressive role for the stroma in PDAC. In the mammary gland, Gustavo Leone's group has shown that genetic inactivation of *Pten* in stromal fibroblasts accelerates the progression of mammary epithelial tumors. In the intestine, Tomi Mäkelä and colleagues have shown that LKB1 signaling in mesenchymal cells is required for suppression of gastrointestinal polyposis, whereas Gijs van den Brink's group showed that Indian Hedgehog signaling from the stroma is required for adenoma formation. Therefore, a much more detailed study of the tumor-suppressive and tumor-promoting aspects of the tumor stroma is required, and this is likely to be tissue-tumor specific. Care should be taken in overinterpreting depletion studies, which may have dramatic effects on homeostasis.

Clearly work remains to reconcile these two new studies, specifically to determine if IKKβ is being deleted in distinct fibroblast populations through the use of different Cre drivers. Furthermore, it is possible that constitutive (ColVICre) versus inducible (Col1a2Cre-ER) deletion of IKKβ could have biological implications, particularly regarding genetic compensation mechanisms through development. Uniform analysis using the same conditionally inducible Cre driver, sampled at the same time point, will be essential here. Most importantly though, these results suggest there is heterogeneity in intestinal fibroblasts and that targeting pathways may have distinct outcomes in different fibroblast populations.

Together, these two studies offer very exciting new insights into NF-κB signaling in stromal fibroblasts in colitis-associated cancer and show that we are only just beginning to understand the complexity of the tumor microenvironment. Understanding the differences between these studies could offer profound new insights into fibroblast heterogeneity in the intestinal epithelium.



Genetic deletion of IKKβ in intestinal mesenchymal cells in the context of colitis-associated tumorigenesis results in either a decrease (Koliaraki et al.) or an increase (Pallangyo et al.) in tumorigenesis, depending on the specific Cre recombinase used.

Koliaraki,V., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20150542

Pallangyo, C.K., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20150576

Robert P. Fordham and Owen J. Sansom, Cancer Research UK Beatson Institute: R.Fordham@beatson.gla.ac.uk and o.sansom@beatson.gla.ac.uk

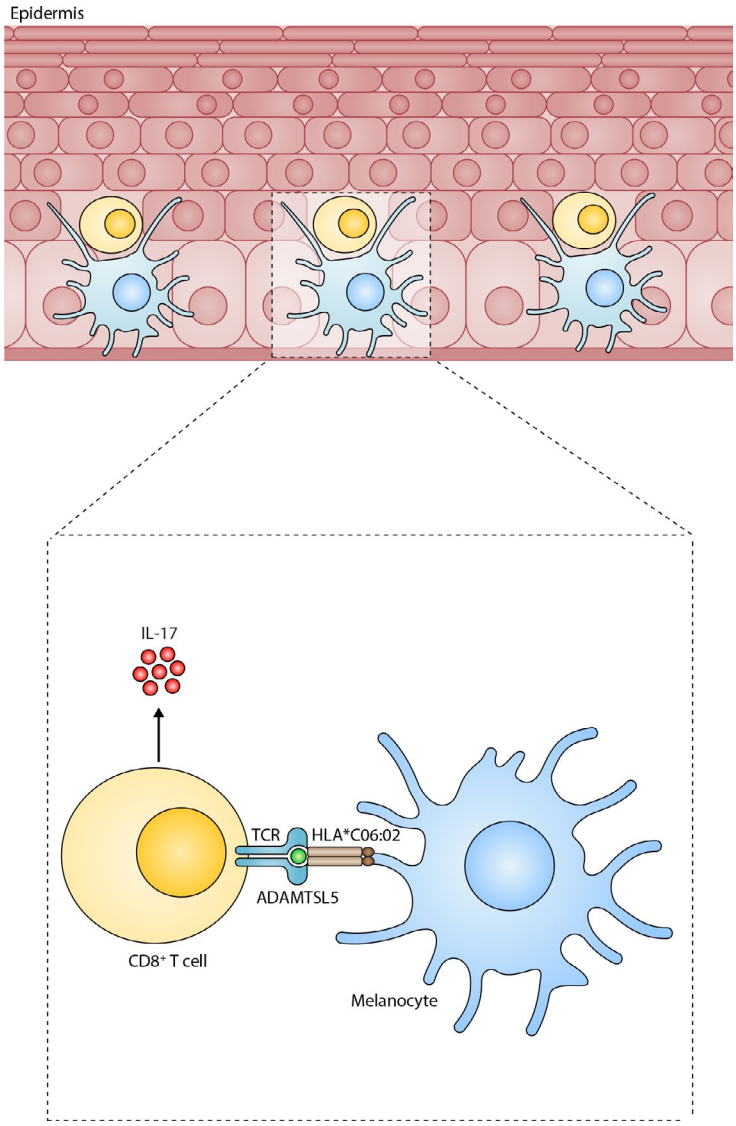
An autoimmune "attack" on melanocytes triggers psoriasis and cellular hyperplasia

A new study by Jörg Prinz's group has identified a melanocyte-derived autoantigen that triggers T cell activation in psoriasis vulgaris.

Insight from James Krueger

For two decades, psoriasis has been classified as a probable autoimmune disease based on a strong disease association with HLA-C*06:02, the ability of T cell–directed therapies to produce disease resolution, and the absence of a known infectious agent or exogenous antigen that triggers the disease. Only recently, there has been the suggestion that cathelicidin (LL37), a keratinocyte-derived antimicrobial peptide might serve as an autoantigen in psoriasis, but not all patients have T cell reactivity to this target.

In this study, a second plausible antigen, ADAMTS-like protein 5 (ADAMTSL5) that is produced by melanocytes is identified as an activating antigen for IL-17–producing T cells that are restricted by HLA-C*06:02. The activation of IL-17–producing T cells is important, as increasing evidence places Th17 and Tc17 cells as the central pathogenic immune cells in psoriasis. Although T cells are found juxtaposed to melanocytes in psoriasis lesions, the type of response that is triggered is not cytotoxic to melanocytes, and in fact, melanocytes are increased in psoriasis lesions, paralleling epidermal hyperplasia that is a key feature of this disease.



In psoriasis, CD8⁺ T cells recognize ADAMTSL5 as an autoantigen presented by melanocytes in HLA-C*06:02 molecules. ADAMTSL5 stimulation triggers the psoriasis signature cytokine, IL-17A.

From the standpoint of autoimmune responses that damage cells and tissues in many other organs, the response in psoriasis is distinctly different, as the immune reaction triggers a wound-healing response pathway in the skin that can resolve with restoration of normal skin structure and function. In part, this may be related to the functions of Th17 and Tc17 cells that, rather than serving as cytotoxic effectors, stimulate and amplify innate immune pathways in target cells. By targeting keratinocytes, IL-17 increases transcription of many antimicrobial proteins, including the autoantigen LL37, and induces the production of chemokines CXCL1, 2, 3, and 8, attracting neutrophils to the site of inflammation. Moreover, CXCL1 may also amplify the autoimmune response because it was first identified as a melanocyte growth factor and might also drive melanocyte and ADAMTSL5 expansion in psoriasis lesions.

This work has some other interesting pathogenic implications. First, the selective growth of melanocytes in skin epithelium may help to explain why psoriasis is largely a skin-restricted disease. Second, the absence of melanocytes from the interfollicular epidermis in most nonhuman species may help to explain why a spontaneous psoriasis-like disease does not occur in lower species.

Arakawa, A., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20151093

James G. Krueger, The Rockefeller University: kruegej@rockefeller.edu

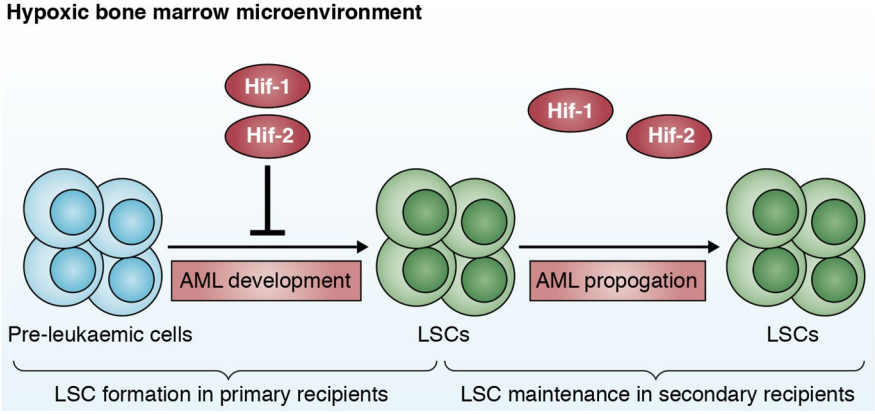
Hypoxia factors suffocate leukemic stem cell initiation

Insight from Mick Bhatia

Acute myeloid leukemia (AML) is the most common form of acute leukemia in adults, with AML deaths in the US reaching nearly 11,000 in 2014. Unfortunately, the clinical outcome of AML is poor, with the majority of patients experiencing relapse within three years. Frequent occurrence of comorbidities precludes treatment with a standard chemotherapy regimen, and the field continues to seek novel therapies that may target leukemic stem cells (LSCs), felt to be the root cause of relapse and refractory disease. However, ideal targets for drug therapies remain elusive.

Hypoxia-inducible factor-1α (HIF-1α) is a subunit of a heterodimeric transcription factor containing a basic helix-loop-helix domain and represents the key transcriptional regulator of cellular and developmental response to low oxygen level (hypoxia). AML is initiated within the bone marrow (BM), likely under local hypoxic conditions. Recently, Dominique Bonnet and colleagues reported that shRNA-based down-regulation of mediators of cellular responses to hypoxia, such as HIF-1α or HIF-2α, induces apoptosis and prevents leukemic engraftment upon transplantation into mice (the functional property that defines LSCs). This observation suggested that HIF-1α or HIF-2α is required for the maintenance of LSCs and may represent potential therapeutic targets for AML. In contrast, the group of Jörg Cammenga has recently shown that genetic deletion of Hif-1α has no effect on mouse AML maintenance, introducing a contradiction in the role of HIFs in AML disease. A complete understanding of the role of HIFs in AML, however, is lacking and calls for sophisticated genetic approaches.

In this issue, Vukovic et al. develop a conditional genetic model to examine the impact of deletion of Hif-2α or both Hif-1α and Hif-2α at different stages of Meis1/Hoxa9- or MLL-AF9–driven leukemogenesis. The authors reveal that Hif-2α suppresses the development of LSCs but has no impact on AML propagation in a Meis1/Hoxa9-induced murine AML model. Hif-2α deletion accelerates LSC development but does not affect LSC maintenance of Mll-AF9–driven AML. The lack of requirement for Hif-2α in AML propagation was surprising; thus, the Kranc group used the recently available CRISPR-Cas9–mediated genome editing approach to determine the potential impact of HIF-2α ablation in human leukemic cells. HIF-2α ablation had no effect on human AML lines, including on cell cycle regulation. In the context of therapeutic targeting, they used BAY 87–2243, a compound able to impair HIF-1α and HIF-2α protein accumulation under hypoxic conditions, to show that inhibition of the HIF pathway has no effect on human AML cell survival or proliferation.



Under hypoxic conditions within the bone marrow, Hif-1α and Hif-2α act in a synergistic manner to suppress the establishment of LSCs from preleukemic cells, thus slowing down AML development. However, once LSCs are established, *Hif-1α* and *Hif-2α* are dispensable for LSC maintenance and leukemia propagation. Therefore, in this proposed model, Hif-1α and Hif-2α play different roles at distinct stages of AML leukemogenesis.

This study represents the first genetic evidence that Hif-2α may act as a tumor suppressor in AML development and/or initiation but is dispensable for LSC-based disease maintenance. Consistent with this idea, the authors show that Hif-1α and Hif-2α deletion promoted a gene expression signature that facilitated survival and proliferation of preleukemic cells and suggest that this is responsible for the selective effects of HIF pathway on leukemic initiation and not on AML established by LSC trasnplantation. Accordingly, this study questions the use of targeting HIFs in AML after critical events of leukemic initiation have occurred.

Before neglecting molecular or pharmacological targeting of HIF in AML, other caveats to these observations need to be explored. Like most drugs, the compound inhibitor used in this study, BAY 87–2243, has many modes of action, including the ability to affect mitochondrial functions. The importance of mitochondrial capacity and AML has been previously demonstrated by the Schimmer group, but it remains to be examined in a conditional deletion model similar to the one used by Vukovic et al. This may provide insights on the role of the HIF pathway and the metabolome of AML LSCs that have yet to be uncovered. Furthermore, as AML is a genetically heterogeneous cancer and is characterized by several molecular alterations, the current studies, like many recent reports for AML disease in vivo, are restricted to the Mll-AF9–Meis1/HoxA9–driven leukemia that represents only a fraction of human AMLs.

Before any conclusions can be drawn, the role of HIFs and their pharmacological inhibition will need to be tested in other clinical models that capture the heterogeneity of patients and their response to chemotherapy. Mouse xenografts of human AML cells may serve as an experimental alternative to test the role of the HIF pathway in leukemia-initiating cells (LICs), using in vitro manipulation of the pathway to quantitatively determine the effects on frequency of transplanted human LICs from a diverse set of AML patients. Because HIFs are known to regulate SDF-1 in the BM niche, it would also be interesting to target this pathway in mice with established human AML and investigate the effect of the tumor microenvironment on LSC function in vivo.

Until then, distinguishing the effects on leukemia initiation versus maintenance by targeting the HIF pathway and demonstrating the selectivity for "preleukemic cells," as opposed to normal HSCs, is where the low-hanging oxygen may lie in the treatment or prevention of AML.

Vukovic, M., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20150452

Mick Bhatia, McMaster Stem Cell and Cancer Research Institute: mbhatia@mcmaster.ca