with constitutive activation of the NF-κB pathway demonstrated B cell and plasma cell hyperplasia, with frequent presence of serum monoclonal M-spikes. Mice with *Blimp1* inactivation demonstrated presence of $CD138^-$, $IRF4^+$, and $BCL6^-$ monoclonal large B cell tumors with highly somatically mutated immunoglobulin genes, consistent with an ABC-like human DLBCL. Similarly, mice with combined constitutive activation of NF-κB and *BLIMP1* inactivation who showed shorter survival also developed B cell lymphomas similar to human ABC-like DLBCL.

These two manuscripts explicitly demonstrate that *BLIMP1* is a bona-fide tumor suppressor gene involved in the pathogenesis of ABC-like DLBCL. They also prove that blocking differentiation is an important step in the pathogenesis of the ABC-like subtype. However, they still do not resolve the enigma of the nature of the precursor cell in this subtype. Furthermore, the study by Calado et al. also clearly demonstrates the synergy in oncogenesis between lesions that block differentiation (BLIMP1) and promote proliferation/survival (NF-κB). However, the low DLBCL penetrance and long latency in these mice suggest that additional transformation events are most probably required for development of the ABC-like tumors. A search in mice tumors for mutations affecting proteins controlling activation of the NF-κB (e.g., A20), commonly found in human ABC-like DLBCL, was negative. Identifying additional pathophysiologic mechanisms contributing to the pathogenesis of DLBCL and generation of drugs specifically targeting the "culprits" will be next important steps.

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Partners in Crime: Genes within an Amplicon Collude to Globally Deregulate Chromatin in Lymphoma

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In this issue of *Cancer Cell*, Rui et al. identify *JAK2* and *JMJDC2* as two contiguous, coamplified oncogenes in primary mediastinal B cell and Hodgkin lymphoma. Together, JAK2 and JMJD2C induce major changes in chromatin structure and gene expression. Targeting these proteins with small molecules represents a new avenue for therapy.

The classification of lymphoma has moved beyond morphology to ever more refined molecular subtypes, with distinct therapeutic implications. Gene expression profiling led to the classification of diffuse large B cell lymphoma (DLBCL) into activated B cell, germinal center B cell, and primary mediastinal B cell lymphoma (PMBL) subtypes (Dave et al., 2006). Profiling also showed that PMBL and Hodgkin's lymphoma (HL) could be grouped together (Rosenwald et al., 2003). Genome-wide copy number analysis coupled with expression profiling reinforced the view that these subtypes are pathogenetically distinct diseases (Lenz et al., 2008). PMBL and HL, in particular, frequently display amplification of chromosomes 2p and 9p. Amplification of *REL* at 2p14-16, which encodes c-Rel (a subunit of NF-κB), induces the constitutive activation of that antiapoptotic pathway.

JAK2 and JMJDC2 were among the genes previously suspected but not proved to have pathogenic roles within the 9p23-24 amplicon. To define the critical genes within the 9p amplicon in

PML and HL, Rui et al. (2010) took a functional approach, creating a library of shRNA molecules targeting each of the overexpressed putative oncogenes within the amplicon. Using a robust screen, they showed that depletion of JAK2 and JMJD2C, as well as the uncharacterized gene RANBP6, was toxic for PMBL and HL but not other lymphoma cells (Rui et al., 2010).

JAK2 is a tyrosine kinase that docks with cytokine receptors and regulates cell proliferation, differentiation, apoptosis, and cell migration in part by phosphorylation of STAT transcription factors. JAK2 gained notoriety because of the presence of constitutive activating mutations of the protein in polycythemia vera and other myeloproliferative neoplasms (MPN). However, the oncogenic effect of JAK2 in PMBL and HL is due to its overexpression, which is associated with constitutive phosphorylation and activation of the kinase. In PMBL, JAK2 hyperactivity was associated with STAT6 activation, IL13 secretion, IL13 receptor expression, and a feed-forward autocrine loop to further support cell proliferation (Rui et al., 2010). Thus, antibodies directed to IL13 represent a way to interfere with the oncogenic program of these cells. Furthermore, PMBL and HL lines were sensitive to JAK2 inhibitors, suggesting that these drugs, currently in clinical trial for MPN, might play a role in the treatment of these specific forms of lymphoma (Rui et al., 2010).

Rui et al. (2010) further show that JAK2 cooperated with both JMJD2C and RANBP6 to support lymphoma growth, with JAK2 knockdown or inhibition leading to cell death and JMJDC2 and RANBP6 depletion to cell cycle arrest. JMJD2C was originally identified as GASC1, a gene amplified in gastric carcinoma, and can be rearranged with the immunoglobulin locus in mucosa associated lymphoma tissue (MALT) lymphoma. JMJDC2 is an iron and 2-oxoglutarate-dependent dioxygenase that catalyzes demethylation of histone 3 on lysine 9 (H3K9) (Cloos et al., 2006). Depletion of JMJD2C from cancer cells as well as murine embryonic stem cells decreased overall trimethylated H3K9 (H3K9me3) levels and cell proliferation and c-*MYC* was identified as one target potentially responsible for this effect (Wang et al., 2010). JAK2 and JMJDC2 converged on a common chromatin mechanism in PMBL and HL. The laboratories of Kouzarides and Green first showed that a fraction of JAK2 enters the nucleus where it phosphorylates the histone H3 on tyrosine 41 (H3Y41), precluding the binding of chromo-shadow domain of heterochromatin protein 1 (HP1) to histone tails (Dawson et al., 2009). HP1 normally facilitates heterochromatin formation by binding to H3K9me3 through its chromo domain and can cause heterochromatin spreading by the attraction of H3K9 methyl transferases G9s, ESET, SETDB1, and Su(Var)39. Increased JMJD2C activity would break this cycle by removing the key H3K9me3 mark. Accordingly, Rui et al. (2010) showed that inhibition or depletion of JAK2 and depletion of JMJD2C in PMBL cells led to a global increase in H3K9me3 levels and foci of HP1 staining.

Rui et al. (2010) demonstrated that JAK2 inhibition and JMJD2C knockdown decreased expression of c-MYC and c-MYC target genes and increased the level of H3K9 trimethylation at the c-MYC promoter (Rui et al., 2010). There was a surprisingly modest effect of JAK2 inhibition on genes containing STAT binding sites, suggesting that direct chromatin phosphorylation by JAK2 and not activation of STATs was the predominant activity of overexpressed JAK2 in PMBL. Supporting this, genome-wide mapping of H3Y41 phosphorylated chromatin identified >2000 genes potentially directly bound and activated by JAK2, including JAK2 itself, JMJD2C, and c-MYC. Given the fundamental role of c-MYC in cancer and embryonic stem cell self-renewal, it is perhaps not surprising that its depletion inhibited the growth of all PMBL and HL cell lines tested. However, a drive to express c-MYC cannot fully explain the oncogenic action of JMJD2C and JAK since c-MYC overexpression could not rescue the effect of JAK2 or JMJDC2 knockdown (Rui et al., 2010). The identities of genes bound and regulated by JMJD2C and/or JAK2 critical to PMBL and HL remain to be determined. Regardless, the autoregulation of JAK2 emphasizes the importance of targeting this protein to break positive reinforcement of aberrant signaling, gene expression, proliferation, and self-renewal.

RANBP6 was the third gene identified in the work of Rui et al. (2010), but its mode of action remains obscure. RANBP6 is over 80% similar to importin 5 (RANBP5), a RAN GTP binding protein implicated in recognition of nuclear localization signals and directional transport of proteins into the nucleus. Overexpression of other importins was associated with an adverse prognosis in cancer (Chahine and Pierce, 2009), and a truncated form of an importin identified in a breast cancer cell line blocked proper nuclear import of p53. It is thus possible that RANBP6 overexpression may lead to aberrant nuclear localization of gene regulators, contributing to malignancy. Whether RANBP6 affects chromatin or gene expression pathways similar to or distinct from those affected by JAK2 and JMJDC2 remains to be determined.

Global epigenetic changes represent an emerging, recurrent theme in cancer. A wide variety of cancers were shown to be deficient in H4K20me3 as well as DNA methylation, although the reason for this remains unclear. Global decreases of H3K27 methylation were associated with a poor prognosis in several malignancies. In many cases, epigenetic changes can be directly linked to cancer genetics. For example, loss of the H3K27me3 modification was associated with inactivating mutations of EZH2 in myeloid malignancy, while increased H3K27me3 was associated with a gain of function mutation of EZH2 in lymphoma (Morin et al., 2010; Sneeringer et al., 2010). Myeloma associated with rearrangement of the WHSC1/MMSET gene displays increased H3K36 methylation and decreased H3K27 methylation, and mutation of SETD2 in renal cell carcinoma was associated with loss of H3K36 methylation. Furthermore, mutations in a DNA methyltransferase DNAMT3a in AML, presumed to be inactivating or dominant negative in nature, were associated with DNA methylation changes, and inactivating mutation of TET2 is associated with global changes in DNA hydroxymethylation and methylation. To this list of disorders associated with widespread chromatin aberration we can add PMBL/HL and JAK2-associated MPN. In the case of the 9p amplicon of PMBL/HL, aberrant expression of two epigenetic modulators at the same time appeared to cooperate in carcinogenesis.

Changes in expression and activity of enzymes regulating chromatin structure upset the normal balance of gene regulation. Which genes change in response to a global insult and whether these disorders all converge on common oncogene

networks, such as that of MYC, remains to be determined. While Rui et al. (2010) showed that JAK2 inhibitors affect chromatin in PMBL and abrogate an oncogenic program, whether these agents affect MPN in the same manner remains to be determined. On the horizon are histone demethylase inhibitors. A JMJDC2 inhibitor was recently identified (Hamada et al., 2010), and it will be critical to test the activity of such agents in PMBL, HL, MPN, and other tumors that harbor the 9p23-24 amplicon.

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New Antibody to Stop Tumor Angiogenesis and Lymphatic Spread by Blocking Receptor Partnering

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Tvorogov et al. (2010) describe in this issue of *Cancer Cell* an antibody that inhibits homodimerization of vascular endothelial growth factor receptor-3 (VEGFR-3) and its heterodimerization with VEGFR-2, but not ligand binding. The work provides mechanistic insights into receptor dimerization and an approach to suppress both angiogenesis and lymphangiogenesis.

More than 800,000 cancer patients worldwide are currently being treated with angiogenesis inhibitors. Treatment with the monoclonal antibody bevacizumab to block vascular endothelial growth factor (VEGF), a cytokine that promotes blood vessel growth, delays progression, and prolongs survival in some cancers (Bagri et al., 2010). Other macromolecular therapeutics that block VEGF signaling, including ramucirumab, an antibody that targets VEGF receptor-2 (VEGFR-2), and aflibercept, a chimeric decoy receptor that binds VEGF, are in advanced clinical trials (http://www.clinicaltrials.gov). These agents are selective, are well tolerated, and generally have only modest side effects restricted to consequences of inhibiting VEGF in normal organs.

However, selective VEGF blockers are efficacious in many cancers only when administered in combination with chemotherapy, and tumors can progress while on therapy. The slowing of tumor growth after inhibition of VEGF signaling can be accompanied by increased invasiveness and metastasis in some preclinical models (Paez-Ribes et al., 2009). The mechanisms of dependence on chemotherapy, progression during treatment, and exaggerated aggressiveness are unclear, but more efficacious approaches are actively being sought.

Receptor-blocking antibodies that target the ligand-binding site of receptors compete with the ligand. This type of inhibitor has the potential limitation of being less efficient at high ligand concentrations, when the ligand out-competes the inhibitor. Because delivery of antibodies to tumors is hampered by inefficient blood vessels, erratic blood flow, and high intratumoral pressure, inhibitors may not reach their molecular targets in sufficient amount and uniformity to be fully efficacious. In addition, other mechanisms contribute to the limitations of efficacy of angiogenesis inhibitors. Factors other than VEGF can promote