functions of most of the type II HDACs. The exception is HDAC6, which is localized primarily in the cytosol and plays important roles in microtubule-mediated trafficking via its ability to promote tubulin deacetylation. Among the sirtuins, most attention has been paid to SIRT1, which is known for its role in promoting longevity, regulating metabolism and autophagy, and modulating the acetylation of several well-known transcription factors (p53, FOXO3A, NFκB-p65, and HSF1). The popular natural product resveratrol inhibits SIRT1, but the HDACis that are currently being evaluated clinically do not. Importantly, the effects of HDAC6 on tubulin acetylation mediate the coordination of protein turnover via the proteasome and autophagy, presumably by facilitating the trafficking of protein aggregates to prelysosomal structures known as “aggresomes.” Thus, HDACis that target HDAC6 or HDAC6 siRNAs disrupt aggresome formation and promote death in MM cells and other cancer cells exposed to PIs (see figure panel A), providing an attractive molecular explanation for the cytotoxic effects of PI–HDACi combinations.

The results of the study by Kikuchi et al in this issue turn this model on its head. The authors showed that bortezomib increased histone acetylation by decreasing expression of HDACs 1-3 (but not HDAC6 or SIRT1) in MM cell lines and primary tumor isolates, and they linked these effects to the synergy observed in cells exposed to bortezomib plus the potent pan–HDAC inhibitor, romidepsin (see figure panel B). Bortezomib’s effects were mediated via caspase-8—dependent cleavage and inactivation of the transcription factor Sp-1, which functions as a potent inducer of type I HDAC expression. Importantly, overexpression of HDAC1 inhibited bortezomib-induced cell death, whereas HDAC1 knockdown promoted cytotoxicity, indicating that HDAC1 inhibition was sufficient to explain the cytotoxic effects observed. The data are reminiscent of earlier findings by Miller et al, who showed that another clinically relevant PI (NPI-0052) increased histone acetylation via a caspase-8—dependent mechanism. A central role for type I HDACs is consistent with data obtained with the type I–selective drug, MS-275 (now SNDX-275), which also synergizes with PIs in hematopoietic tumor cells even though it does not inhibit HDAC6.

In the end, given that both classes of drug are “dirty,” the molecular mechanisms involved in their cytotoxic effects are likely to be very complicated and context-dependent. There is still good reason to be attracted to the “proteotoxicity” model (see figure panel A) given the work emerging from the neurodegenerative disease literature and the fact that the immediate target of proteasome inhibitors is protein degradation. Within this context, PI-induced, caspase-8—dependent HDAC inhibition could reinforce type I HDAC-dependent processes that contribute to cell killing. Furthermore, given the broad role Sp-1 and its relatives play in driving gene expression, the effects of bortezomib should extend well beyond the type I HDACs to include a variety of other survival-associated transcriptional targets. It could be argued that the true test of both models will be whether they can help identify the subsets of patients who would benefit most from combination therapy. For example, the proteotoxicity model predicts that sensitivity might be linked to baseline ER stress, rates of protein synthesis (translation), and/or the efficiency of nonproteosomal degradative pathways (autophagy), whereas in the HDAC inhibition model sensitivity might be linked to type I HDAC (and particularly HDAC1) levels. Fortunately, both models can now be tested. In the end, as is most often the case in biology, the answer will probably involve a combination of the 2 models plus mechanisms we have not yet identified.

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Comment on Steidl et al, page 418

Genomic imbalances in Hodgkin lymphoma

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Hodgkin lymphoma is still in many aspects enigmatic. In this issue of Blood, Steidl and colleagues present exciting novel insights into pathogenesis and clinical behavior of classical Hodgkin lymphoma by performing array comparative genomic hybridization studies to detect genomic gains and losses in the HRS tumor cells of this malignancy.

The treatment of Hodgkin lymphoma is a success story with about 80% to 90% of patients reaching long-term cure with current treatment strategies. Nevertheless, about 10% to 20% of patients cannot be cured and will die of the disease. Therefore, there is much interest in identifying characteristics of resistant tumors upfront. Patients with such tumors may benefit from an early treatment intensification, whereas patients with a good prognosis may benefit from reduced treatment intensity and...
consequently less toxic side effects of the chemo- and/or radiation therapy.2

Prognostic markers can be identified in various ways. An attractive approach is to search for genetic lesions in the tumor cells that are associated with good or bad clinical outcome. This track was followed by Steidl et al, who performed a high-resolution array-based comparative genomic hybridization study with isolated Hodgkin and Reed-Sternberg (HRS) cells from 53 cases of classical Hodgkin lymphoma. The cases included 23 patients in whom the disease progressed or relapsed at any time. Multiple recurrent genomic gains and losses were found, including several known from other works. Interestingly, one of the gains, involving the chromosomal region 16p11.2-13.3, was significantly associated with the group of treatment failures. Thus, the authors present here the first genetic feature of HRS cells that may serve as a prognostic marker for Hodgkin lymphoma. Clearly, this finding needs validation in an independent group of homogeneously treated patients. Moreover, a weakness of the study is that the treatment failure group included patients with disease progression and relapse at any time. In clinical practice, patients who do not respond to treatment at all or have an early relapse within several months after therapy have a worse prognosis than patients with a relapse after several years.3 Likewise, as the reasons for relapse may be different in those with early versus late recurrence, it will be important to find out whether the 16p gain helps to identify the therapy resistant group. Preliminary findings in the study indicate that 16p gains may indeed be most frequent in therapy refractory patients.

As we still have only a fragmentary knowledge of the transforming events involved in Hodgkin lymphoma pathogenesis, it is another aim of the study to identify novel recurrent imbalances that may point to oncogenes or tumor suppressor genes contributing to Hodgkin lymphoma development. Most genetic lesions identified in HRS cells affect the NF-κB pathway, including MAP3K14 (NIK), IKBKB, and NFκB. The detection of recurrent gains of the NF-κB gene is also of interest in a further regard. HRS cells have largely lost the expression of B cell–specific genes, but a small fraction of such genes remain expressed.4 These include key components for an interaction of B cells with T cells, that is, CD80, MHC class II, and CD40.3 The interaction of HRS cells with surrounding T helper cells through these stimulating pathways presumably promotes their survival and proliferation. Thus, in a fraction of Hodgkin lymphomas gains of the CD40 gene may contribute to the high expression of CD40 by HRS cells in a setting where most B-cell typical genes are down-regulated, and thereby have a pathogenetic effect.

Among the many genes located in the large gained region on 16p, the authors turned their interest to one specific gene, ABC1, which is a member of the ATP binding cassette (ABC) transporter family and encodes an efflux pump that functions as a multidrug resistance factor. One Hodgkin lymphoma line, KMH2, also had a gain of the ABC1 locus, and inhibition of the high ABC1 expression in KMH2 cells sensitized them to killing by doxorubicin. Hence, ABC1 is an attractive candidate for a pathogenetically relevant gene in the gained region on 16p. This finding is of particular interest also in light of another recent study, which identified small subpopulations of so-called “side population cells” in 2 other Hodgkin lymphoma cell lines, L428 and HDLM2 (KMH2 was not analyzed).5 Side population cells are characterized by their exclusion of the Hoechst dye 33342, due to expression of multidrug resistance genes.5 The side population cells of the L428 and HDLM2 lines expressed the ABC transporter family members ABCB1 (MDR1) and ABCG2 and were more resistant to chemotherapy than the nonside population cells.6 Although the patterns and mechanisms of ABC transporter expression appear to be different in KMH2 versus L428 and HDLM2 cells, both studies nevertheless point to a potentially important

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![Diagram of NF-κB pathway and ABC1 expression](image)

Main findings of the study and their potential consequences are summarized. Recurrent gains (indicated by an additional gene copy on one allele) of 16p11.2-13.3 include the ABC1 gene. This may lead to increased ABC1 expression, and the increased expression of this multidrug transporter may cause chemoresistance of the lymphoma cells. Increased gene copy numbers for components of the NF-κB pathway, including MAP3K14 (NIK) and IKBKB, may cause increased expression of these positive regulators of NF-κB activation and consequently contribute to the strong constitutive NF-κB activity in HRS cells. The NF-κB pathway is displayed in a simplified way, not discriminating the canonical and noncanonical pathway. The CD40 gains may contribute to strong CD40 expression in a setting where most B cell–typical genes are down-regulated. CD40 promotes the interaction of HRS cells with surrounding CD40 ligand-expressing T cells and likely also contributes to NF-κB activity in HRS cells.
role of these molecules in chemotherapy resistance and treatment failure in Hodgkin lymphoma. Importantly, as side population cells share features with cancer stem cells, these findings will certainly also stimulate further work to elucidate potential relationships among expression of multidrug transporters, chemotherapy resistance, and putative HRS lymphoma “stem” cells.10

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Comment on Kohli et al., page 456

New era dawns on sickle cell pain

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In a commendable study in this issue of Blood, Kohli and colleagues describe a novel approach to unraveling the complexities of the pathogenesis of sickle cell pain and its management.1 Given the timing of this publication, it can be considered a celebratory centennial of the discovery of sickle cell disease in the United States in 1910.2

Sickle cell anemia is almost synonymous with pain. Acute painful episodes are its hallmark and the most common cause of hospitalization.3 Vaso-occlusion is believed to be the root cause of the pain; it causes damage to tissues supplied by the occluded vessel and is also responsible for creating a state of chronic vascular inflammation that explains many features of sickle cell pain.4,4 Tissue damage and vascular inflammation generate a number of inflammatory mediators that initiate an electrical impulse of pain transmitted along peripheral nerves (Aδ and C fibers) to the dorsal horn of the spinal cord. The impulse ascends along the contralateral spinothalamic tracts to the thalamus, which interconnects reversibly with other centers, most notably with the limbic system (mediator of emotion and memory). At the same time, the central nervous system (CNS) inhibits the transmission of the painful stimulus at the level of the dorsal horn via a descending pathway, with norepinephrine and serotonin as neurotransmitters, which begins in the periaqueductal gray matter of the midbrain. Eventually the modified electrophysiological impulse that started at the site of vaso-occlusion is sent to the cerebral cortex, where it is perceived as pain. Pain perception is thus a subjective phenomenon and is the result of a complex interplay among enhancing and inhibiting factors at the level of the CNS in addition to a host of coexisting psychosocial and environmental factors. Given the subjective nature of sickle pain coupled with the lack of experimental models to investigate, it generated an atmosphere of doubt about its authenticity and the reliability of the patients’ complaints. The negative attitudes toward patients with painful sickle crises have been compounded by racial stereotypes, the effects of the disease in limiting educational and employment opportunities, suboptimal medical coverage, and the large doses of opioids often required to obtain pain relief. Unlike other types of pain, research support and progress in understanding sickle cell pain followed a sluggish, slothful, almost stagnant, path. In a sense, it has been a situation of retrograde translational medicine working in reverse, where lack of evidence created a barrier to rational clinical management. Hydroxyurea decreases the frequency of painful episodes but it is not an analgesic to treat acute pain directly.2

In this issue of Blood, Kohli et al take advantage of a transgenic sickle cell mouse model expressing human sickle hemoglobin to characterize the behavioral, neurochemical, and pharmacologic aspects of sickle cell pain as well as to find alternatives to opioid analgesics to treat pain.2 The mice used were the homozygous and hemizygous Berkley strain (BERK and hBERK1), compared with control mice expressing human hemoglobin A (HbA-BERK). Determinants of behavioral change included reduced paw withdrawal threshold to mechanical stimuli, reduced withdrawal latency to thermal stimuli, and decreased grip force in both homozygous and hemizygous mice indicating musculoskeletal and cutaneous hyperalgesia. At the neurochemical level, peripheral nerves and blood vessels were structurally altered in BERK and hBERK1 skin with decreased expression of mu opioid receptors and increased calcitonin gene-related peptide and substance P immunoreactivity. The reduction in innervations is indicative of peripheral neuropathy that may culminate in a central neuropathic pain condition. Similarly, activators of neuropathic and inflammatory pain were increased in the spinal cord of hBERK1 compared with HbA-BERK. These neurochemical changes in the periphery and spinal cord are suggestive of nociceptor and glial activation that may contribute to hyperalgesia in mice, similar to the characteristics of pain observed in patients with sickle cell anemia. Taken together, these findings suggest that the characteristics and severity of sickle cell pain depend on the location, extent, and chronicity of the neurochemical damage that ensues after vaso-occlusion. Thus, depending on whether the damage is
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