In a cell culture system, the three compounds were found to increase the amount and activity of β-hexosaminidase in fibroblasts obtained from patients with ISD and ATSD. Analysis of the mode of action of the inhibitors revealed that they most likely function by stabilizing the enzyme, and thus qualify to be called pharmacological chaperones. Interestingly, the observed increase in activity (3-fold in the best case) would be predicted to confer sufficient function to have beneficial effects in patients, making them good candidates for the development of clinically useful drugs. The three novel pharmacological chaperones displayed better selectivity profiles than the known β-hexosaminidase inhibitor that was previously shown to have pharmacological chaperone activity [8], leading to the hope that they could have fewer undesirable off-target effects. Also of significant interest, some of the identified compounds share chemical scaffolds with drugs that have already been approved by the FDA, increasing the likelihood that they could meet the criteria for good drug candidates.

In addition to identifying novel pharmacological chaperones with therapeutic potential for the treatment of Tay-Sach diseases, the present study represents a proof of principle that high-throughput assays can be used to identify new chemical entities with pharmacological chaperone activity—a path that will undoubtedly be followed by investigators in search of novel therapeutic avenues for treating conformational diseases. Although the present screening campaign was searching for inhibitors, there are no a priori theoretical reasons why other types of ligands (agonists, allosteric regulators, etc.) that bind and stabilize misfolded proteins could not also act as pharmacological chaperones. Thus, high-throughput screens based on the ability of compounds to restore normal subcellular targeting, independent of their intrinsic signaling activities, are also likely to be carried out in the near future.

Upcoming and ongoing clinical trials for Tay-Sachs disease and other lysosomal storage disorders such as Fabry disease will soon tell us if the initial clinical results for pharmacological chaperones [7] can be generalized. It will be interesting to see if screening for novel pharmacological chaperones will become a common approach in the search for conformational disease treatments.

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Reprogramming the Histone Code

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Histone lysine methyltransferases, like G9a, play central roles in the regulation of gene expression. A current study by Kubicek et al. [1] reports the identification of a G9a small-molecule inhibitor, thereby opening the way to new epigenetic cancer therapies.

An important part of the pathogenesis of cancer lies in the inactivation of tumor suppressor genes, which can be achieved either genetically or epigenetically. Epigenetic alterations refer to changes in gene expression that do not result from alterations in the DNA sequence. Epigenetic mechanisms include DNA methylation, and histone protein acetylation and methylation. Both DNA methylation and histone acetylation have been the target of small-molecule therapies [2, 3], while the development of compounds that target lysine and arginine methyltransferases has lagged behind. In a recent issue of *Molecular Cell*, Kubicek et al. [1] described screening for and identification of a highly specific, small molecular weight histone lysine methyltransferase (HKMT) inhibitor that

perturbs the levels of the histone H3 lysine 9 dimethyl-mark (H3K9me2), which is a signature of transcriptional repression. The bunazosin-related compound selectively inhibits the HKMT G9a, but not the closely related HKMT GLP. This compound also does not inhibit the H3K9me3 HKMT SUV39H1, the H3K9me1 HKMT SET7/9, or the primary arginine methyltransferase PRMT1.

Global inhibitors of methylation do exist and are used as research tools. These include analogs of *S*-adenosylmethionine (AdoMet), the methyl donor, like sinefungin and methylthioadenosine, and small molecules that inhibit *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase like adenosine dialdehyde (AdOx) and 3-deaza-neplanocin A (DZNep), which cause intracellular accumulation of AdoHcy and feedback inhibition of most methylation reactions. These broad-spectrum inhibitors lack the finesse of molecules that can target the action of a single enzyme, and are thus of limited utility in studies on target biology or as leads for molecularly targeted therapeutics. Recently, high-throughput screens (HTS) were performed to identify inhibitors of specific protein methyltransferases, including PRMT1 [4] and SU(VAR)3-9 [5]. Although these studies identified inhibitory molecules, in both cases the HTS hits were not optimal. While the identified arginine methyltransferase inhibitors (AMIs) did not inhibit HKMTs, they lacked specificity within the PRMT family (i.e., these AMIs inhibited all the PRMTs tested). The isolated SU(VAR)3-9 inhibitor is a fungal compound called chaetocin. Chaetocin also inhibits a subset of other SET domain-containing HKMTs, including DIM5 and G9a, albeit at slightly higher concentrations. Chaetocin can form disulfide bonds with many intracellular proteins, limiting its utility as a tool for studying the biology of SU(VAR)3-9 [6]. In addition, it is a relatively large molecule (MW = 696.84 Da), hence limiting its potential for chemical optimization toward SU(VAR)3-9 and other HKMTs.

It is at this juncture that Kubicek et al. [1] enter the picture with their screen for G9a inhibitors, which would be expected to perturb the histone H3 lysine 9 methylation states. They performed an HTS with 125,000 compounds, preselected on the basis of similarity to a pharmacophore fingerprint built with the AMI compounds [4]. The screen used the N-terminal tail of histone H3 as a substrate and the SET domain of G9a fused to GST as the enzyme. The screen was performed at a high AdoMet concentration (20 μM) to limit the number of "hits" that would likely be AdoMet competitive. AdoMet-competitive compounds would most likely inhibit other AdoMet-utilizing enzymes, including DNA methyltransferases, isoprenylcysteine carboxylmethyltransferases, protein L-isoaspartyl *O*-methyltransferases, and PRMTs [7].

The discovery of a highly specific inhibitor, the bunazosin (α-adrenoreceptor antagonist) analog BIX-01294, highlights the success of the screen and the possibility that other existing drugs and their analogs could hit the HKMT family. A viable approach to find drug-like inhibitors for other HKMTs would thus be to screen for HKMT side activities of such compounds. Another identified inhibitor, BIX-01338, was competitive with AdoMet and inhibited several HKMTs nonspecifically; however, it could still potentially be amenable to chemical optimization to hone selectivity toward different HKMTs in a manner similar to what has been achieved with ATP-competitive inhibitors of kinases [8, 9]. Moreover, the *d*issociation *e*nhanced *l*anthanide *f*luorescence *i*mmuno-*a*ssay (DELFIA) [10] format used for G9a will be relatively easy to replicate with other histone methyltransferases, provided the right peptide substrate and matching methyllysine-specific antibody are utilized. An isotopic assay format [11] and an S-adenosylhomocysteine hydrolase-coupled assay [12] have been reported previously and are also generally applicable to any protein methyltransferase.

A G9a small-molecule inhibitor is clearly of pharmaceutical interest. G9a and its homolog GLP were found to copurify with the transcriptional corepressor protein CtBP [13]. That same study showed that siRNA knockdown of CtBP, G9a, or GLP resulted in upregulation of E-cadherin in cancer cells where this gene was epigenetically downregulated. Downregulation of E-cadherin and other epithelial genes by transcriptional repressors, such as ZEB1 and ZEB2, that recruit CtBP and G9a/GLP is a hallmark of the epithelial-mesenchymal transition that underlies the progression of cancer to metastasis [14]. Moreover G9a has been implicated in the function of CutL1 [15]. CutL1 is a transcription factor that activates a transcriptional program regulating genes involved in cell motility, invasion, and extracellular matrix composition downstream of TGFbeta signaling [16]. Chemical inhibitors of G9a could thus potentially contribute to upregulation of E-cadherin and attenuation of CutL1 function with the hopes of impeding the shift to metastasis.

Many other HKMTs have been linked to human disease and could serve as promising targets for small-molecule drug therapy. Such is the case with EZH2, a histone H3 lysine 27 HKMT overexpressed in many aggressive cancers where it is predictive of poor outcome [17]. Inhibition of EZH2 by siRNA has been shown to prevent metastasis of PC-3 prostate cancer cells in mice [18]. Moreover, there is accumulating evidence to implicate HKMTs such as Smyd3, SETDB1, NSD1, NSD2, NSD3, and Suv39h1 in human cancers [19, 20].

The discovery of a small molecule that can specifically inhibit G9a is a great accomplishment, which shows for the first time that this class of epigenetic regulator is a viable target for drug development efforts. Similar screens to that conducted by Thomas Jenuwein's group [1] could be used to identify small-molecule inhibitors for other HKMTs and hence herald a promising new wave of molecularly targeted therapeutics for cancer and other diseases. Such agents would also increase the arsenal for combination with existing epigenetic (HDAC and DNMT inhibitors) and signal transduction drugs (e.g., kinase inhibitors).

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