Chromatin-targeting small molecules cause class-specific transcriptional changes in pancreatic endocrine cells

Chromatin-targeting small molecules cause class-specific transcriptional changes in pancreatic endocrine cells

Stefan Kubicek[a,b,c], Joshua C. Gilbert[b], Dina Fomina-Yadlin[a,b,d], Alexander D. Gitlin[b,1], Yuan Yuan[a,b,d], Florence F. Wagner[b], Edward B. Holson[b], Tuoping Luo[b], Timothy A. Lewis[b], Bradley Taylor[b], Supriya Gupta[b], Alykhan F. Shamji[b], Bridget K. Wagner[b], Paul A. Clemons[b], and Stuart L. Schreiber[a,b,e,2]

[a]Howard Hughes Medical Institute and [b]Broad Institute of Harvard and MIT, Cambridge, MA 02142; [c]CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, A-1090 Vienna, Austria; and [d]Department of Molecular and Cellular Biology and [e]Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138

Contributed by Stuart L. Schreiber, February 3, 2012 (sent for review November 10, 2011)

Under the instruction of cell-fate–determining, DNA-binding transcription factors, chromatin-modifying enzymes mediate and maintain cell states throughout development in multicellular organisms. Currently, small molecules modulating the activity of several classes of chromatin-modifying enzymes are available, including clinically approved histone deacetylase (HDAC) and DNA methyltransferase (DNMT) inhibitors. We describe the genome-wide expression changes induced by 29 compounds targeting HDACs, DNMTs, histone lysine methyltransferases (HKMTs), and protein arginine methyltransferases (PRMTs) in pancreatic α- and β-cell lines. HDAC inhibitors regulate several hundred transcripts irrespective of the cell type, with distinct clusters of dissimilar activity for hydroxamic acids and orthoamino anilides. In contrast, compounds targeting histone methyltransferases modulate the expression of restricted gene sets in distinct cell types. For example, we find that G9a/GLP methyltransferase inhibitors selectively up-regulate the cholesterol biosynthetic pathway in pancreatic but not liver cells. These data suggest that, despite their conservation across the entire genome and in different cell types, chromatin pathways can be targeted to modulate the expression of selected transcripts.

histone modification | gene regulation | chemical epigenetics | beta cell biology | cholesterol pathway

Epigenetic mechanisms mediated through chromatin control cell-state and cell-type decisions during development and in the adult (1, 2). Chromatin-modifying enzymes have been shown to function by interacting with master transcription factors, regulating the expression of key target genes and conferring epigenetic memory through the propagation of modifications to the chromatin template. These modifications include methylation of the DNA itself and a variety of posttranslational modifications to histones, the proteins most closely interacting with DNA. Depending on the type of modification, e.g., acetylation or methylation, and the exact position of the modified amino acid, these modifications can activate or repress transcription of the underlying DNA sequence (3).

Small molecules targeting chromatin have mainly been developed for the treatment of cancer, justified by the identification of genetic aberrations leading to overexpression or activation of several chromatin-modifying enzymes (4–7). In contrast to clinically approved HDAC and DNMT inhibitors, fewer compounds targeting histone lysine methyltransferases (HKMTs) and protein arginine methyltransferases (PRMTs) are available. These small molecules are mainly used as chemical probe compounds in basic research, and toxicity is often limiting for testing in animal models. Interestingly, for most of the 50 HKMTs and 30 histone demethylases encoded in the human genome, no specific compounds are available. Even for HDACs, most compounds inhibit HDACs 1, 2, 3, and 6 (8), and HDAC8-specific compounds are only now emerging (9).

Chromatin-modifying enzymes play important roles in normal development, supported by the early embryonic lethality of animal knockout models for many of these proteins. We hypothesize that modulating chromatin pathways can also affect cell types and cell states in the adult. Recent studies have shown that overexpression of master regulatory transcription factors can cause the reprogramming of differentiated cell types to induced pluripotent stem cells and the transdifferentation to other lineages (10). In the pancreas, it has been shown that overexpression of the transcription factor *Pax4* converts glucagon-expressing α cells into insulin-producing β cells (11). Modulating the expression levels of the endogenous factors with small molecules therefore has the potential to induce similar changes without the need to deliver transgenic sequences of potentially oncogenic proteins under the control of powerful promoters. During pancreatic development, it has been shown that treatment with HDAC inhibitors changes the ratio of endocrine cell types dependent on whether a hydroxamic acid or orthoamino anilide compound is used (12). The close developmental origin and epigenetic plasticity between α and β cells make these cell types an interesting model system for studying the effects of chromatin-targeted compounds.

To identify a broader set of target genes that can be regulated by modulating the activities of chromatin-modifying enzymes, we measured the genome-wide transcriptional effects of 29 compounds in pancreatic α- and β-cell lines. The results indicate that compounds cause similar effects independent of the cell line in which they were profiled. All clinical HDAC inhibitors fell into the structural classes of hydroxamic acids and orthoamino anilides, respectively, and up- and down-regulated hundreds of transcripts. In contrast, more selective compounds like the HKMT inhibitor BIX-01294 have specific effects. We show that treatment with BIX-01294 leads to the selective up-regulation of the entire cholesterol biosynthetic pathway, correlating with increased cholesterol levels and reduced hormone secretion in these pancreatic cell lines.

Results

We selected 29 compounds targeting different classes of chromatin-modifying enzymes, including 22 HDAC inhibitors, three DNMT inhibitors, one PRMT inhibitor, and three HKMT inhibitors (*SI Appendix*, Fig. S1). On the basis of the large amount

Author contributions: S.K., A.F.S., B.K.W., P.A.C., and S.L.S. designed research; S.K., D.F.-Y., A.D.G., Y.Y., B.T., and S.G. performed research; F.F.W., E.B.H., T.L., and T.A.L. contributed new reagents/analytic tools; S.K., J.G., and P.A.C. analyzed data; and S.K., A.F.S., B.K.W., P.A.C., and S.L.S. wrote the paper.

The authors declare no conflict of interest.

Freely available online through the PNAS open access option.

Database deposition: The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE36379).

[1]Present address: Weill Cornell/Rockefeller/Sloan-Kettering Tri-Institutional MD-PhD Program, New York, NY 10065.

[2]To whom correspondence should be addressed. E-mail: stuart_schreiber@harvard.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1201079109/-/DCSupplemental.

of previous work on HDACs, our compound set is enriched for small molecules targeting this enzyme class. In addition to the approved drug SAHA, we included other hydroxamic acid HDAC inhibitors: ITF-2357, PXD101, CRA-024781, and LBH-589, all currently in clinical development (13); the widely used tool compounds trichostatin A (TSA), scriptaid, and pyroxamide; and active SAHA analogs BRD-K17311666, BRD-K22912318, BRD-K90919562, and BRD-K92415738. Benzamides, containing an orthoamino anilide biasing element and thereby forming a chemically distinct class of HDAC inhibitors were represented by CI-994, MS-275, and MCGD-0103, which are all currently in clinical development, and by the research tool compounds BRD5298, BRD6929 (compound 60) (14), BRD9773, and BRD8451. Furthermore, cyclic peptide HDAC inhibitors apicidin and HC toxin and the ketone BRD-A94377914 (15) were included.

To target other classes of chromatin-modifying enzymes, compounds were chosen that have mostly been previously described: as HKMT inhibitors, we used chaetocin (16), a nonspecific compound originally described as selective for SU(VAR)3–9 and later shown to inhibit G9a (17) and thioredoxin reductase (18); BIX-01294 (19, 20), a G9a/GLP-specific compound, and its active analog BRD-K62233722. DNMT inhibitors were 5-aza-deoxycytidine (decitabine) (21), zebularine (22), and RG-0108 (23). AMI-1 (24) was used as a PRMT inhibitor.

For expression profiling, we wanted to select a concentration that allows detecting compound effects while avoiding nonspecific cytotoxicity signatures. Therefore, we first measured the dose-dependent effects of compounds on cellular ATP levels after 24-h treatment. We then selected a concentration of each compound corresponding to 80% preservation of ATP levels in the more sensitive cell line. For compounds that did not display toxicity, we chose a maximum concentration of 10 μM (*SI Appendix*, Table S1). We then measured gene expression changes in mouse α- and β-cell lines αTC1 and βTC3 induced by treatment with these compounds for 1, 6, and 24 h (Fig. 1*A*).

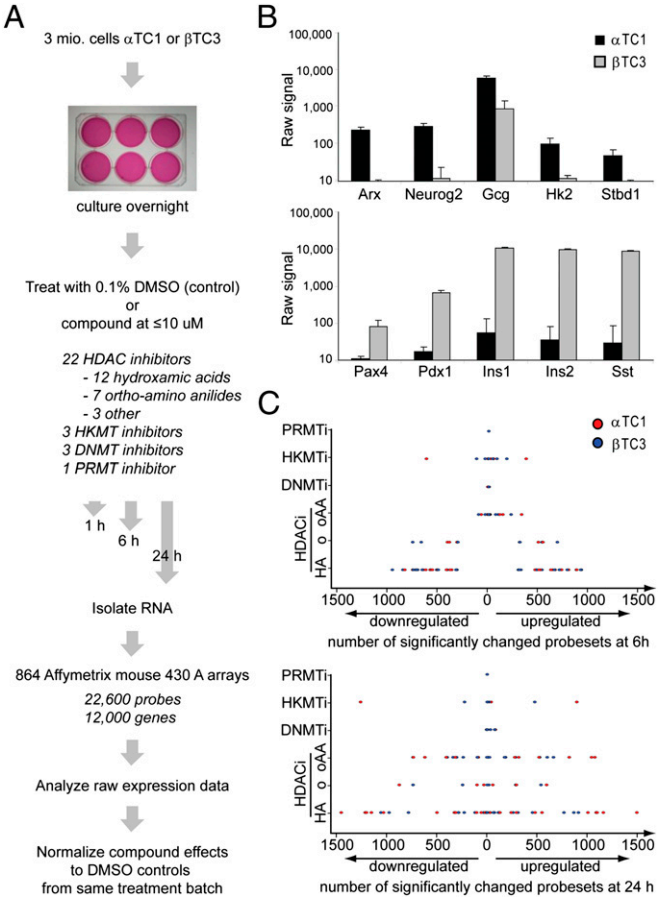

Fig. 1. α and β cells have distinct gene-expression signatures. (*A*) Experimental outline of expression profiling in mouse pancreatic endocrine cell lines. (*B*) Known cell-type–specific marker genes are differentially expressed in the expected patterns. Means and SDs of raw expression values of all DMSO-treated controls are indicated for α-cell–specific (*Upper*) and β-cell–specific (*Lower*) genes. (*C*) Number of probe sets with significantly changed expression summarized by compound class. For HDAC inhibitors (HA, hydroxamic acids; o, others; oAA, orthoamino anilides), DNMT inhibitors, HKMT inhibitors, and the PRMT inhibitor, the number of significantly and more than twofold changed probe sets are plotted at 6 h (*Upper*) and 24 h (*Lower*) of compound treatment.

Expression Changes Are Compound-Specific and Similar Across Different Cell Types. We first analyzed the dataset for differences between DMSO-treated α and β cells used as controls and identified 15,734 out of 22,716 probe sets to have altered expression levels (*SI Appendix*, Fig. S2). These data indicate that the α- and β-cell lines used are significantly different in baseline gene-expression patterns, despite being of closely related developmental origins and derived with an identical strategy of SV40 large T antigen overexpression (25, 26). Compared with expression profiles of mouse cell lines derived from other tissues (27, 28), α cells are approximately two- to threefold more similar to β cells than to bone marrow dendritic cells or embryonic stem cells (*SI Appendix*, Fig. S2*B*). Importantly, many of the known α- and β-cell–specific genes, including key transcription factors and hormones, are differentially expressed between the two cell types (Fig. 1*B*). In addition to these well-studied transcription factors, other DNA-binding proteins are differentially regulated between the two cell types and may prove essential for approaches aimed at transdifferentiating α to β cells (*SI Appendix*, Tables S2 and S3).

To reduce the number of probe sets in the subsequent analyses, we performed ANOVA and LIMMA (29, 30) and removed probe sets that were not changed under any of the treatment conditions. We first analyzed the raw expression data and observed clustering exclusively with cell type and not with compound treatment or time point (*SI Appendix*, Fig. S3*A*). These data indicate that after 1, 6, or 24 h of compound treatment, α-cell expression patterns still largely resemble those of DMSO-treated α cells and treated β cells are closest to β-cell controls. We then normalized all raw data to DMSO controls matched for cell type, treatment batch, and time point. For every compound treatment we calculated the fold change over a sample, or mean of two samples, treated with DMSO at the same time and processed identically. We then $\log_2$ transformed these fold changes and averaged the three biological replicates. Interestingly, these data no longer exclusively cluster with cell type (*SI Appendix*, Fig. S3 *B* and *C*). Rather, certain compounds induce similar changes in both α- and β-cell lines. For example, transcriptional changes induced by the HDAC inhibitor SAHA are highly correlated between α and β cells (*SI Appendix*, Fig. S4*A*). Importantly, this observation not only holds true between mouse α and β cells, but also with expression profiles collected in the Connectivity Map (31) for human cell lines. When we used the genes most significantly changed by HDAC inhibitors in α and β cells as input, the connectivity map predicted SAHA as the compound most likely to cause such a transcriptional change in human cancer cells. That transcriptional targets of compound treatment are conserved in different species and cell types is further supported by the correlation between the expression changes caused by SAHA in α cells, β cells, and human MCF7 cells (*SI Appendix*, Fig. S4).

Similar Numbers of Transcripts Are Up- and Down-Regulated. We next analyzed the different compounds for their global patterns of transcriptional effects. To identify probe sets that were significantly changed upon compound treatment, we first used a modified Student *t* test to determine significant differences in raw signal for each probe set between the three replicates of compound-treated states and all matched DMSO controls for that

time point and cell line. For significantly altered probe sets, we further considered those with a more than twofold change in the DMSO-normalized signal. In general, we observed very few expression changes at the 1-h time point (*SI Appendix*, Fig. S5). At the 6-h time point, 17 compounds already induce hundreds of transcripts. Interestingly, for most compounds the number of up-regulated transcripts approximately equals the number of repressed genes. HDACs are predominantly associated with repressive functions; therefore, we expected direct targets to be up-regulated after inhibitor treatment. Rather, we observe that hydroxamic acid HDAC inhibitors up-regulate on average 550 probe sets 6 h after compound addition and down-regulate approximately equal numbers of genes. Although we cannot be certain that the down-regulated transcripts we observe are direct targets of HDAC-mediated activation, the appearance of gene-expression changes within 6 h after HDAC inhibitor treatment, and the low number of changes at 1 h after treatment, are consistent with locus-specific repressive roles of histone acetylation. These results correlate with previous findings, including activating roles for HDACs in yeast (32) and mammalian cells (33), and extend these data by excluding compensatory effects following genetic manipulations and prolonged compound treatment.

For the number of transcripts affected, we observe clear differences dependent on the targeted enzyme class and chemical structure of the inhibitors (Fig. 1*C*). HDAC inhibitors of the hydroxamic acid and cyclic peptide classes regulate ∼1,100 probe sets, representing more than 600 transcripts, after 6 h. In contrast, the orthoamino anilide-containing HDAC inhibitors regulate only 100 transcripts at the 6-h time point, of which most are up-regulated. At 24 h, orthoamino anilides regulate as many transcripts as hydroxamic acids but still cause more up- than down-regulation. In contrast, some hydroxamic acid-based HDAC inhibitors lose effects over time and regulate significantly fewer transcripts at 24 h than at 6 h.

Whereas HDAC inhibitors regulate several hundred transcripts, DNMT inhibitors, HKMT inhibitors, and the PRMT inhibitor regulate many fewer transcripts. Only the toxic compound chaetocin, which in addition to HKMTs also inhibits thioredoxin reductase, has widespread effects. Compounds targeting DNA methyltransferases only show strong transcriptional effects at the 24-h time point, when they induce the DNA-damage response and proapoptotic genes. The PRMT inhibitor AMI-1 does not cause a greater than twofold expression change of any transcript.

Chromatin-Targeted Compounds Regulate Transcripts Essential for Pancreatic Cell Function and Identity. To detect expression changes essential for β-cell function, we examined compound effects at the latest time point (24 h). We first selected all genes involved in glucose-stimulated insulin secretion and clustered compounds by the effects they have on that gene set in β cells (*SI Appendix*, Fig. S6). HDAC inhibitors cause the most pronounced effects and we observe up-regulation of potassium voltage-gated channel *Kcnb1*, syntaxin 1a (*Stx1a*), and synaptosomal-associated protein 25 (*Snap25*) and repression of the ATP-sensitive potassium channel Kir6.2 encoded by gene *Kcnj11* and the voltage-dependent calcium channel *Cacna1a*. These data suggest a complex effect of HDAC inhibitors on insulin secretion, with some essential components of the pathway up- and others down-regulated.

We then determined the effects of chromatin-targeted compounds on the expression of the 190 differentially regulated transcription factors in α cells at 24 h (*SI Appendix*, Fig. S7*A*). We were particularly interested in whether any of the compounds caused effects indicative of cell-type conversion by activation of β-cell transcription factors and repression of α-cell–specific markers. Compared with all other compounds, the HKMT inhibitor chaetocin has a very distinct profile of activities. Importantly, it represses α-cell marker genes *Arx* and *Brn4*, and activates β-cell–specific master regulatory transcription factors *Pax4* and *Nkx6.1* (*SI Appendix*, Fig. S7*B*). Similarly to chaetocin, most HDAC inhibitors positively regulate the expression of β-cell factors and repress many α-cell factors. Functionally, this results in the up-regulation of insulin and other β-cell–specific markers (*SI Appendix*, Fig. S8) in α cells, whereas few pancreas-specific markers are up-regulated when β cells are treated with these compounds (*SI Appendix*, Fig. S9).

Two Classes of HDAC Inhibitors. Both chaetocin and HDAC inhibitors have widespread effects beyond pancreatic marker genes. The unbiased clustering of expression data from all samples (Fig. 2*A*) revealed two major transcriptional responses corresponding to chemically distinct classes of HDAC inhibitors consisting of hydroxamic acids and orthoamino anilides. Interestingly, the cyclic peptides apicidin and HC toxin and the ketone BRD-A94377914 cluster tightly with hydroxamic acids. We identified the marker genes regulated by HDAC inhibitors in general and for the specific classes, and used gene set enrichment analysis (GSEA) (34, 35) to find the apoptotic pathway as most discriminating between the HDAC inhibitor classes (*SI Appendix*, Fig. S10). Particularly, at the 6-h time point orthoamino anilides but not hydroxamic acids, down-regulate proapoptotic genes *Bad*, *Bip*, *Casp9*, *Ikbkb*, and *Nfkbib*, and increase the expression of antiapoptotic genes like *Phlda1*.

Importantly, the effects of the two classes of HDAC inhibitors on pancreatic master regulatory transcription factors and marker genes are very different (Fig. 2*B*): Hydroxamic acids cause the down-regulation of β-cell markers (*Pax4*, *Pdx1*, *Isl1*, *Insm1*, *NeuroD1*, *Nkx6.1*, and *Nkx2.2*) and α-cell marker (*Arx*). β-Cell markers are mostly unaffected by orthoamino anilides, which rather cause a slight up-regulation of α-cell markers (*MafB* and *Arx*), resulting in significantly increased glucagon expression in β cells at 24 h (*SI Appendix*, Fig. S10).

Interestingly, BRD-K04466929 and unique orthoamino anilides BRD-K28115298, BRD-K37439773, and BRD-K68338451 do not cluster with the clinical compounds CI-994, MS-275, and MCGD-0103. Whereas the clinical compounds inhibit HDAC1, -2, and -3 at approximately equal concentrations in the 10–100 nM range (8), these compounds are selective for only HDAC1 and -2 and only inhibit HDAC3 at 100-fold higher concentrations. At both the 6-h and 24-h time points, these compounds regulate fewer transcripts than the clinical orthoamino anilides, suggesting that their higher biochemical selectivity is also reflected in more specific gene-expression responses.

Specific HKMT Inhibitor BIX-01294 Causes Selective Up-Regulation of the Cholesterol Biosynthesis Pathway. Whereas the nonspecific and toxic HKMT inhibitor chaetocin regulates similar numbers of genes compared with HDAC inhibitors, the more specific G9a/GLP HKMT inhibitors BIX-01294 and BRD-K62233722 regulate very few transcripts. When we performed GSEA for these probe sets, we observed specific and highly significant up-regulation of the cholesterol biosynthetic pathway (*SI Appendix*, Fig. S11). At both 6 h and 24 h, most genes in the pathway were up-regulated more than twofold in α cells by BIX-01294 (Fig. 3*A* and *SI Appendix*, Fig. S12). Importantly, this set of genes includes HMG-CoA synthase and HMG-CoA reductase, the rate-limiting enzyme in the pathway (Fig. 3*B*). To test whether the up-regulation of the pathway is G9a/GLP dependent, we applied chromatin immunoprecipitation (ChIP). G9a/GLP is known to catalyze the formation of H3K9me2 at its target genes, and we detect high levels of this modification at the promoters of *Hmgcs1* and *Hmgcr* in DMSO-treated α cells. BIX-01294 treatment reduces the abundance of H3K9me2 at these promoters and the promoter of known G9a target gene *mageA2* (Fig. 3*B*). At the *Hmgcr* promoter, reduction of H3K9me2 correlates with the accumulation of H3K4me3, a modification strongly associated with transcriptional activation. To probe the functional consequence of transcriptional up-regulation of the cholesterol pathway, we measured cellular cholesterol levels in α cells after 48 h of BIX-01294 treatment (Fig. 3*C*) and we observed strong increases in the staining intensity of filipin, a dye that binds nonesterified cholesterol (36).

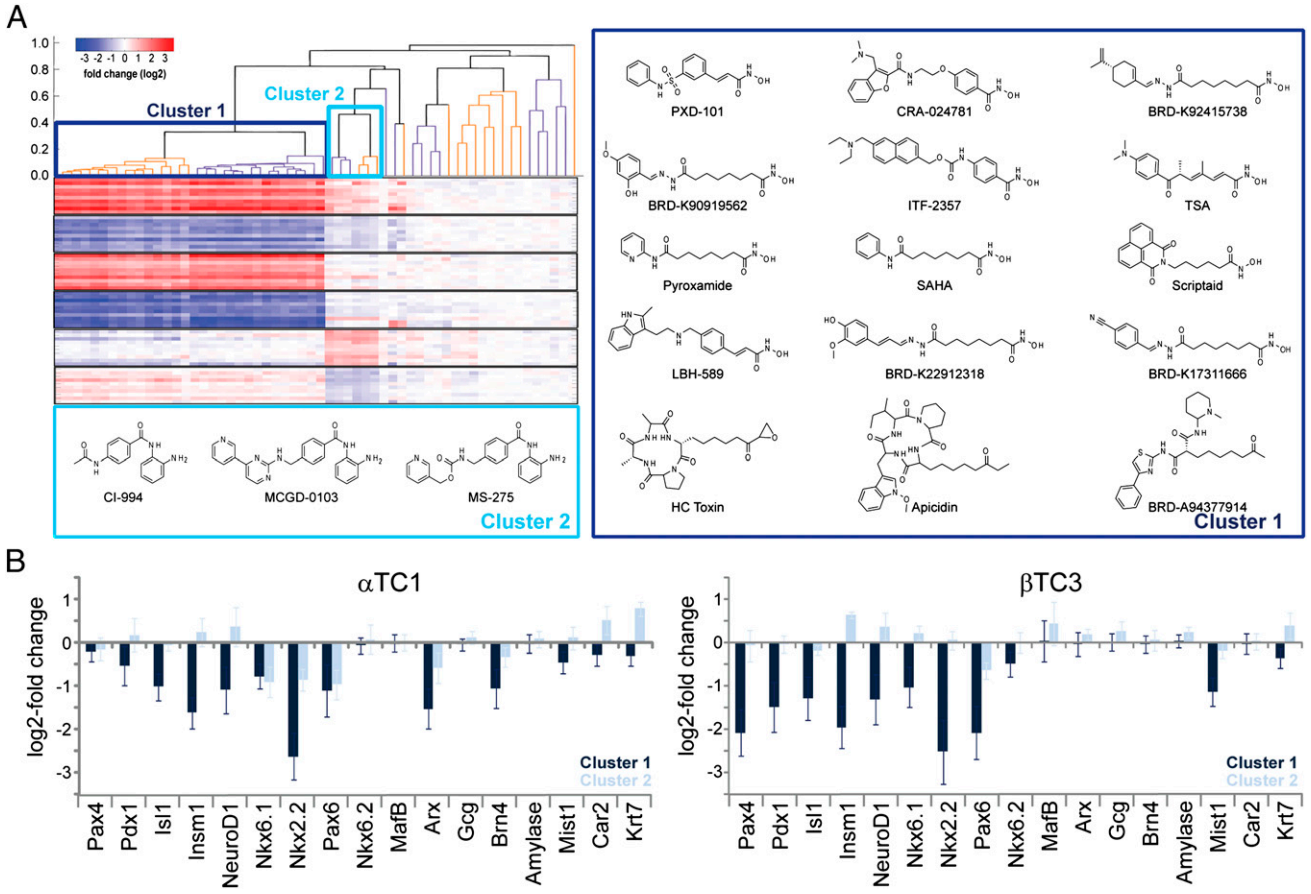

Fig. 2. Different chemical classes of HDAC inhibitors cause distinct transcriptional responses. (*A*) Unbiased clustering of transcriptional changes caused by compounds normalized to DMSO at the 6 h time point identifies similar effects across cell lines. Different activities are observed for compounds in the hydroxamic acid (cluster 1) and orthoamino anilide clusters (cluster 2). (*B*) Expression changes of key pancreatic marker genes and master regulatory transcription factors differ by HDAC compound class. Shown are means and SDs of 45 samples treated with HDAC inhibitors from cluster 1 and 9 samples treated with cluster 2 compounds at the 6-h time point.

Similar to α cells, β cells respond to BIX-01294 treatment by up-regulation of the entire cholesterol biosynthesis pathway (*SI Appendix*, Fig. S13*A*). This up-regulation is also observed in PANC-1 cells of pancreatic ductal origin, where expression of several pathway genes, including *Hmgcs1*, is increased, although to a lesser degree than in endocrine cells. Interestingly, HepG2 cells of hepatic origin do not activate the cholesterol biosynthesis pathway in response to BIX-01294 treatment (*SI Appendix*, Fig. S14). Liver cells might not respond to BIX-01294 treatment with increased expression of cholesterol pathway genes because these are already four- to eightfold more highly expressed in HepG2 cells compared with pancreatic endocrine cells, consistent with the liver being a prime tissue of cholesterol biosynthesis.

H3K9me2 levels are also reduced at the β-cell promoters of *Hmgcs1* and *Hmgr* following BIX-01294 treatment, suggesting direct G9a/GLP-dependent regulation (*SI Appendix*, Fig. S13*B*). To further confirm that these effects are mediated by direct inhibition of G9a/GLP, we used small molecules with different inhibition profiles to BIX-01294. BRD-K62233722, an active analog of BIX-01294 that inhibits G9a with an IC_{50} of ∼10 μM, causes similar but weaker up-regulation of the cholesterol pathway. Furthermore UNC0638 (37), a BIX-01294 analog with increased potency and reduced toxicity, causes even stronger up-regulation of cholesterol pathway genes than BIX-01294 and almost complete loss of H3K9me2 at the promoters of these genes (*SI Appendix*, Fig. S15).

Functionally, elevated cholesterol levels have been linked to decreased β-cell viability and insulin secretion (38, 39). Therefore, we tested insulin secretion in βTC3 cells treated with different concentrations of BIX-01294 (*SI Appendix*, Fig. S13*C*). Consistent with a detrimental effect of high cellular cholesterol levels on insulin secretion, we observe that BIX-01294 reduces insulin secretion in a dose-dependent manner at concentrations that do not impact β-cell morphology (*SI Appendix*, Fig. S13*D*). Increased cholesterol levels likely mediate this reduction of insulin secretion, because BIX-01294 treatment does not significantly alter the expression of genes involved in the insulin secretion pathway (*SI Appendix*, Fig. S6).

Discussion

Chromatin-modifying enzymes target DNA itself (DNA methylation) or histones, the proteins most tightly associated with DNA in the form of nucleosomes, and have therefore been associated with transcriptional regulation. The activities of these enzymes can be modulated with chromatin-targeted compounds. In contrast to genetic methods, small-molecule inhibitors allow studying time-and dose-dependent effects of altered modification levels in the presence of unchanged protein complexes. Consistent with the tight link between chromatin modifications and transcriptional control, we observe strong and rapid effects on gene expression in response to treatment with small molecules that target HDACs and methyltransferases.

Expression studies of compounds inhibiting chromatin-modifying enzymes have focused on HDAC inhibitors in cancer cells (33, 40–43), including the approved drugs vorinostat and romidepsin. Whereas compounds showing selectivity for single proteins out of the 11 human class I and class II HDAC homologs

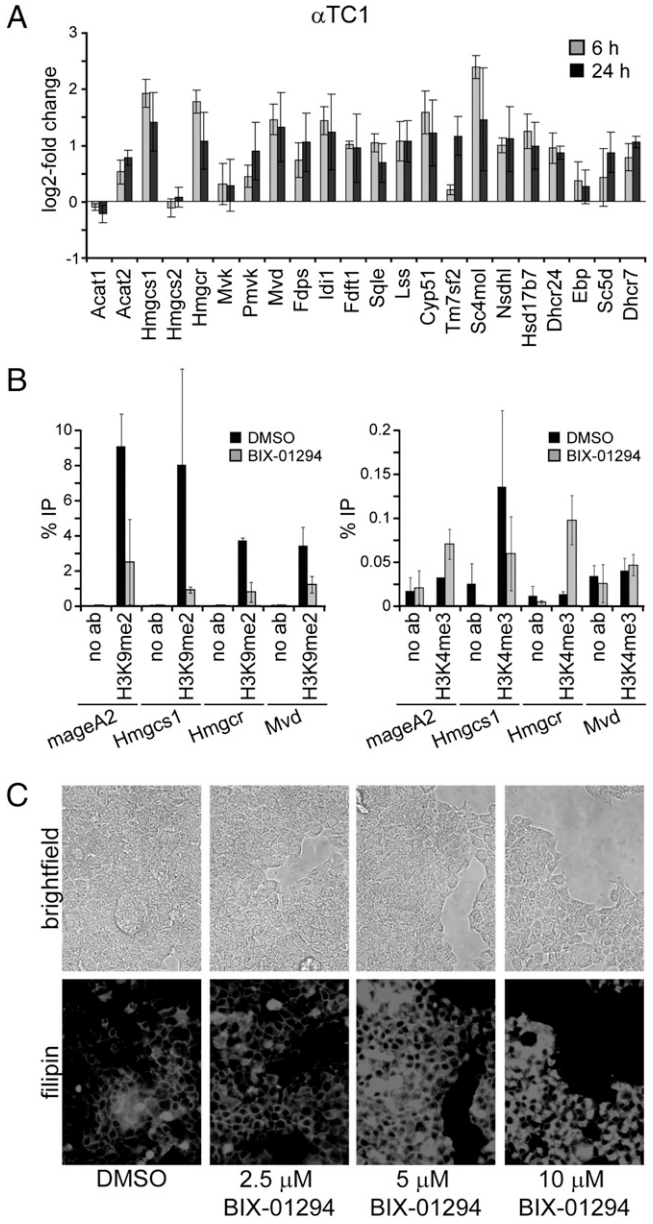

Fig. 3. Specific regulation of the cholesterol biosynthetic pathway by G9a/GLP inhibitor BIX-01294. (*A*) Relative $\log_2$-fold expression changes of cholesterol pathway genes in BIX-01294–treated α cells. For genes that are represented by multiple probe sets on the Affymetrix array, the probe set with the highest raw expression signal in α cells is plotted. (*B*) Chromatin immunoprecipitation at the promoters of cholesterol pathway genes with antibodies directed against H3K9me2, the product of the G9a/GLP HKMTs and H3K4me3, an activating histone modification. (*C*) Increased cholesterol staining in α cells following BIX-01294 treatment. Following 2-d compound treatment, α cells were stained for cholesterol with filipin. (Scale bar, 50 μm.)

have remained elusive, the currently available compounds show wide chemical variability. Biochemical profiling has revealed inhibition of HDAC1, -2, -3, -6, and -8 by most hydroxamic acid HDAC inhibitors, whereas the activity of orthoamino anilides is more restricted to HDACs 1, 2, and 3 (8). Consistently, we observe clustering of the gene-expression responses to these compounds that correlate with their chemical structure classes and biochemical specificities. Different gene-expression changes have previously been described for the comparison of two hydroxamic acids (SAHA and TSA) with an orthoamino anilide (MS-275) in two cell lines (41). Our data extend that observation to 15 more compounds and show that cyclic peptides HC toxin and apicidin, which are related to the approved drug FK228, cluster tightly with hydroxamic acid inhibitors. In contrast, the clinical orthoamino anilides CI-994, MS-275, and MGCD-0103 form a distinct cluster. Therefore, we observe only two main clusters of transcriptional response to these well-studied HDAC inhibitors. For pancreatic genes, these two classes have been known to cause different effects during development (12). We observe that hydroxamic acids repress most endocrine master regulatory transcription factors in mature α and β cells, inconsistent with increased formation of endocrine cells in hydroxamic-acid–treated ex vivo pancreata. However, we cannot rule out proproliferative effects on an endocrine progenitor or incomplete differentiation in organ culture. In contrast, our gene expression data excellently fit the increased formation of α and PP cells in orthoamino-anilide–treated pancreata. The increased transcription of Arx, Brn4, and glucagon already starting at 6 h suggests that these genes might be direct targets of orthoamino anilide HDAC inhibitors.

All HDAC inhibitors regulate hundreds of transcripts; in contrast, HKMT inhibitors cause much more restricted transcriptional responses. The G9a/GLP inhibitor BIX-01294, which selectively increases expression of genes involved in the cholesterol biosynthetic pathway, best exemplifies this trend. Interestingly, G9a has been shown to bind directly the orphan nuclear receptor small heterodimer partner (SHP), a regulator of the bile acid synthesis pathway that uses cholesterol as a substrate (44, 45). G9a catalyzes the formation of H3K9me2, a chromatin modification associated with repression of transcription; therefore upregulation of target genes following the inhibition of G9a is consistent with a direct mechanism. A direct role of G9a in repressing the cholesterol biosynthetic pathway is further supported by the loss of H3K9me2 at the promoters of these genes following BIX-01294 treatment and the even stronger effects of UNC0638, a G9a/GLP inhibitor with higher specificity. Currently, no studies are available describing the role of G9a in pancreatic cell lines. We hypothesize that in pancreatic cells, other G9a target genes are repressed by additional epigenetic mechanisms, e.g., DNA methylation and additional histone modifications, so that a G9a inhibitor alone cannot activate their transcription.

Nucleosomal packaging is a general mechanism used genome-wide in all cell types, but chromatin-modifying enzymes are often bound to cell-type–specific transcription factors or components of the RNA polymerase machinery. Therefore, compound-induced changes could in theory be dependent on the transcriptional program already in place in a particular cell type and vary widely among different cell types, affecting thousands of genes or only small subsets of the transcriptome. Our data suggest a model that discriminates two classes of chromatin-targeted compounds (*SI Appendix*, Fig. S16). Nonspecific compounds that target multiple members of an enzyme class like hydroxamic acid HDAC inhibitors or chaetocin cause expression changes of thousands of genes, presumably by genome-wide disruption of chromatin structure and global changes of modification levels at multiple sites. For these inhibitors, we observe good correlation of compound-induced changes between α and β cells, but even an unrelated human breast cancer cell line responds similarly. In contrast, BIX-01294 inhibits only G9a/GLP, two HKMTs with the same activity for generating H3K9me2. G9a/GLP inhibitors also cause global histone modification changes, but only affect a single site, H3K9me2, whereas HDAC inhibitor treatment increases acetylation at almost all modifies lysines. Genome-wide reduction in H3K9me2 by G9a/GLP inhibition results in the modulation of only a small set of genes, almost all in the cholesterol biosynthesis pathway. This regulation is conserved between α, β, and ductal cells, but hepatic cells do not respond with increased cholesterol pathway expression, suggesting a cell-type–specific response. Highly selective compounds like BIX-01294 and nonspecific HDAC inhibitors represent extremes of transcriptional activities ranging from cell-type–dependent regulation of small sets of genes to conserved transcriptome-wide changes. Intermediates are

possible, and the unique orthoamino anilides with HDAC1/2 selectivity might be the first examples of such compounds.

In summary, our data show that inhibiting chromatin-modifying enzymes with small molecules does not always cause dramatic changes on a transcriptome-wide scale. Rather, selective compounds can activate very specific pathways, making the development of novel small molecules for additional enzymes a high priority.

Materials and Methods

Cell lines αTC1 and βTC3 were treated with compounds in low-glucose DMEM with a final concentration of 0.1% DMSO for 1, 6, or 24 h. RNA was prepared using Qiagen RNeasy kits and hybridized to Affymetrix HT Mouse 430A peg arrays. Raw data were Robust Multichip Average-normalized using GenePattern (46) and analyzed for significant changes using ANOVA and LIMMA (29, 30). Detailed methods can be found in *SI Appendix, SI Materials and Methods*.

ACKNOWLEDGMENTS. We thank Hua Miao and Rebecca L. Maglathlin for compound synthesis; Gregory Wendel, Joshua A. Bittker, Stephen Johnston, and the entire Broad Institute Compound Management team for providing quality-controlled compound samples; Krystal Matthews, Paula Morais, and Kristin Ardlie (Broad Institute Biological Samples Platform) for sample tracking, RNA preparation, and quality control; Jennifer Moran and Andrew Crenshaw (Broad Institute Genetic Analysis Platform) for support and management of Affymetrix experiments; and the entire Chemical Biology Diabetes team for helpful discussion and advice. Funding for this project was provided by the Juvenile Diabetes Research Foundation; the National Cancer Institute's Initiative for Chemical Genetics, National Institutes of Health, under Contract N01-CO-12400; Ernst Schering Research Foundation and European Union FP7 Marie Curie Grant PIOF-GA-2008-221135 (to S.K.); a Molecules, Cells, and Organisms Training Grant from Harvard University (to D.F.-Y.); Type-1 Diabetes Pathfinder Award DP2-DK083048 from the National Institute of Diabetes and Digestive and Kidney Diseases (to B.K.W.); and National Institute of General Medical Sciences Grant GM38627 (to S.L.S.). S.L.S. is an Investigator at the Howard Hughes Medical Institute.

1. Jenuwein T, Allis CD (2001) Translating the histone code. *Science* 293:1074–1080.
2. Schreiber SL, Bernstein BE (2002) Signaling network model of chromatin. *Cell* 111: 771–778.
3. Bernstein BE, Meissner A, Lander ES (2007) The mammalian epigenome. *Cell* 128: 669–681.
4. Chi P, Allis CD, Wang GG (2010) Covalent histone modifications—miswritten, misinterpreted and mis-erased in human cancers. *Nat Rev Cancer* 10:457–469.
5. Marks PA (2007) Discovery and development of SAHA as an anticancer agent. *Oncogene* 26:1351–1356.
6. Campas-Moya C (2009) Romidepsin for the treatment of cutaneous T-cell lymphoma. *Drugs Today (Barc)* 45:787–795.
7. Issa JP, Kantarjian HM (2009) Targeting DNA methylation. *Clin Cancer Res* 15: 3938–3946.
8. Bradner JE, et al. (2010) Chemical phylogenetics of histone deacetylases. *Nat Chem Biol* 6:238–243.
9. Tang W, Luo T, Greenberg EF, Bradner JE, Schreiber SL (2011) Discovery of histone deacetylase 8 selective inhibitors. *Bioorg Med Chem Lett* 21:2601–2605.
10. Zhou Q, Melton DA (2008) Extreme makeover: Converting one cell into another. *Cell Stem Cell* 3:382–388.
11. Collombat P, et al. (2009) The ectopic expression of Pax4 in the mouse pancreas converts progenitor cells into alpha and subsequently beta cells. *Cell* 138:449–462.
12. Haumaitre C, Lenoir O, Scharfmann R (2008) Histone deacetylase inhibitors modify pancreatic cell fate determination and amplify endocrine progenitors. *Mol Cell Biol* 28:6373–6383.
13. Marks PA, Xu WS (2009) Histone deacetylase inhibitors: Potential in cancer therapy. *J Cell Biochem* 107:600–608.
14. Methot JL, et al. (2008) Exploration of the internal cavity of histone deacetylase (HDAC) with selective HDAC1/HDAC2 inhibitors (SHI-1:2). *Bioorg Med Chem Lett* 18: 973–978.
15. Jones P, et al. (2006) A series of novel, potent, and selective histone deacetylase inhibitors. *Bioorg Med Chem Lett* 16:5948–5952.
16. Greiner D, Bonaldi T, Eskeland R, Roemer E, Imhof A (2005) Identification of a specific inhibitor of the histone methyltransferase SU(VAR)3-9. *Nat Chem Biol* 1:143–145.
17. Iwasa E, et al. (2010) Total synthesis of (+)-chaetocin and its analogues: Their histone methyltransferase G9a inhibitory activity. *J Am Chem Soc* 132:4078–4079.
18. Tibodeau JD, Benson LM, Isham CR, Owen WG, Bible KC (2009) The anticancer agent chaetocin is a competitive substrate and inhibitor of thioredoxin reductase. *Antioxid Redox Signal* 11:1097–1106.
19. Kubicek S, et al. (2007) Reversal of H3K9me2 by a small-molecule inhibitor for the G9a histone methyltransferase. *Mol Cell* 25:473–481.
20. Chang Y, et al. (2009) Structural basis for G9a-like protein lysine methyltransferase inhibition by BIX-01294. *Nat Struct Mol Biol* 16:312–317.
21. Jabbour E, Issa JP, Garcia-Manero G, Kantarjian H (2008) Evolution of decitabine development: Accomplishments, ongoing investigations, and future strategies. *Cancer* 112:2341–2351.
22. Yoo CB, Cheng JC, Jones PA (2004) Zebularine: A new drug for epigenetic therapy. *Biochem Soc Trans* 32:910–912.
23. Brueckner B, et al. (2005) Epigenetic reactivation of tumor suppressor genes by a novel small-molecule inhibitor of human DNA methyltransferases. *Cancer Res* 65: 6305–6311.
24. Cheng D, et al. (2004) Small molecule regulators of protein arginine methyltransferases. *J Biol Chem* 279:23892–23899.
25. D'Ambra R, Surana M, Efrat S, Starr RG, Fleischer N (1990) Regulation of insulin secretion from beta-cell lines derived from transgenic mice insulinomas resembles that of normal beta-cells. *Endocrinology* 126:2815–2822.
26. Hamaguchi K, Leiter EH (1990) Comparison of cytokine effects on mouse pancreatic alpha-cell and beta-cell lines. Viability, secretory function, and MHC antigen expression. *Diabetes* 39:415–425.
27. Amit I, et al. (2009) Unbiased reconstruction of a mammalian transcriptional network mediating pathogen responses. *Science* 326:257–263.
28. Stadtfeld M, et al. (2010) Aberrant silencing of imprinted genes on chromosome 12qF1 in mouse induced pluripotent stem cells. *Nature* 465:175–181.
29. Smyth GK (2005) Limma: Linear models for microarray data. *Bioinformatics and Computational Biology Solutions Using R and Bioconductor*, eds Gentleman RC, Carey VJ, Huber W, Irizarry RA, Dudoit S (Springer, New York), pp 397–420.
30. Smyth GK (2004) Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol* 3:Article 3.
31. Lamb J, et al. (2006) The Connectivity Map: Using gene-expression signatures to connect small molecules, genes, and disease. *Science* 313:1929–1935.
32. Bernstein BE, Tong JK, Schreiber SL (2000) Genomewide studies of histone deacetylase function in yeast. *Proc Natl Acad Sci USA* 97:13708–13713.
33. Dejligbjerg M, et al. (2008) Differential effects of class I isoform histone deacetylase depletion and enzymatic inhibition by belinostat or valproic acid in HeLa cells. *Mol Cancer* 7:70.
34. Mootha VK, et al. (2003) PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet* 34: 267–273.
35. Subramanian A, et al. (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA* 102: 15545–15550.
36. Jacobs NL, et al. (1997) Analysis of a Chinese hamster ovary cell mutant with defective mobilization of cholesterol from the plasma membrane to the endoplasmic reticulum. *J Lipid Res* 38:1973–1987.
37. Vedadi M, et al. (2011) A chemical probe selectively inhibits G9a and GLP methyltransferase activity in cells. *Nat Chem Biol* 7:566–574.
38. Fryirs M, Barter PJ, Rye KA (2009) Cholesterol metabolism and pancreatic beta-cell function. *Curr Opin Lipidol* 20:159–164.
39. Hao M, Head WS, Gunawardana SC, Hasty AH, Piston DW (2007) Direct effect of cholesterol on insulin secretion: A novel mechanism for pancreatic beta-cell dysfunction. *Diabetes* 56:2328–2338.
40. Ellis L, et al. (2008) Histone deacetylase inhibitor panobinostat induces clinical responses with associated alterations in gene expression profiles in cutaneous T-cell lymphoma. *Clin Cancer Res* 14:4500–4510.
41. Glaser KB, et al. (2003) Gene expression profiling of multiple histone deacetylase (HDAC) inhibitors: Defining a common gene set produced by HDAC inhibition in T24 and MDA carcinoma cell lines. *Mol Cancer Ther* 2:151–163.
42. Miyanaga A, et al. (2008) Antitumor activity of histone deacetylase inhibitors in non-small cell lung cancer cells: Development of a molecular predictive model. *Mol Cancer Ther* 7:1923–1930.
43. Peart MJ, et al. (2005) Identification and functional significance of genes regulated by structurally different histone deacetylase inhibitors. *Proc Natl Acad Sci USA* 102: 3697–3702.
44. Boulias K, Talianidis I (2004) Functional role of G9a-induced histone methylation in small heterodimer partner-mediated transcriptional repression. *Nucleic Acids Res* 32: 6096–6103.
45. Fang S, et al. (2007) Coordinated recruitment of histone methyltransferase G9a and other chromatin-modifying enzymes in SHP-mediated regulation of hepatic bile acid metabolism. *Mol Cell Biol* 27:1407–1424.
46. Reich M, et al. (2006) GenePattern 2.0. *Nat Genet* 38:500–501.