Synthetic Transcription Activators | Hot Paper

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A Synthetic Small Molecule for Targeted Transcriptional Activation of Germ Cell Genes in a Human Somatic Cell**

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In nature, coordinated genetic and/or epigenetic mechanisms govern the global transcriptional reprogramming that dictates cell fate.[1] In contrast to genetic modifications, which cause irreversible changes of the cellular phenotype, epigenetic modifications can be reversed. The plasticity of epigenetic modifications facilitates the development of novel drugs, including small molecules that are capable of restoring incurable dysfunctions associated with faulty transcriptional machinery.[2] Chromatin-modifying enzymes act both as facilitators and barriers by switching the transcriptional networks "on" and "off" to modulate the chromatin topology that governs the cellular phenotype.[3] In particular, chromatin remodeling mediated by histone acetylation could induce global changes in the transcriptional status of a cell.[4] Accordingly, small molecules that could inhibit histone deacetylase (HDAC), a chromatin-modifying enzyme, were shown to induce global changes in the acetylation profile, but in a non-selective manner.[5] The development of small molecules that trigger targeted transcriptional activation could ensure better efficacy and a reduction of long-term side effects. However, precise control of gene expression is difficult, as epigenetic modifications are not insulated events. Therefore, epigenetically active small molecules with a DNA recognition domain are required. *N*-methylpyrrole (P) and *N*-methylimidazole (I) polyamides are able to recognize each of the four Watson–Crick base pairs by binding to the minor groove of the DNA.[6] As a novel chemical approach to dictate cell fate through targeted transcriptional activation, we have developed a small molecule that has access to both the genetic and epigenetic environment. This small molecule, namely SAHA-PIP, contains the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) and hairpin pyrrole–imidazole polyamides (PIPs), which are capable of sequence-specific DNA recognition.[7] In mouse fibroblasts, we first demonstrated the remarkable ability of SAHA-PIPs to induce differential activation of pluripotent stem-cell-associated genes and fine-tuned these molecules for enhanced efficacy.[7b,8] Likewise, SAHA-PIP-mediated targeted transcriptional activation may trigger unusual activation of the silent gene network(s) in somatic cells.

Gametogenesis is one of those silent biological processes in somatic cells. Meiosis is a highly specialized cell-division process in multicellular eukaryotes that is specific to germ cells.[9] Aberrations in the orderly meiotic process are a prominent cause of human infertility.[10] In mammalian spermatogenesis, the meiotic phase involves a series of intricate processes, such as chromosome remodeling and genetic recombination.[11] Male mammalian germ cells express distinct populations of Piwi-interacting RNAs to govern the silencing of transposable elements in the germline at the pre-pachytene and pachytene stages of meiosis.[12] MOV10L1, a germ-cell-specific putative RNA helicase, functions upstream of Piwi proteins to maintain post-meiotic genome integrity.[13] A recent study revealed that the epigenetic disruption of the PIWI pathway could be associated with spermatogenic disorders in infertile male human patients.[14] As SAHA-PIPs selectively activate a set of pluripotency genes in mouse fibroblasts, they may have a similar effect in human dermal fibroblasts (HDFs) to modulate the typically conserved gene network that is associated with pluripotency and/or gametogenesis. Herein, we report the remarkable ability of the SAHA-PIP **K**, which was developed as part of a SAHA-PIP library (**A** to **Φ**), to trigger targeted transcriptional activation of germ-cell-specific and PIWI-pathway genes in HDFs.

Global changes in gene transcription were analyzed using a SurePrint G3 Human GEv2 8×60K Microarray (Agilent Technologies) after treating a library of synthetic SAHA-PIPs (**A**–**Φ**) with HDFs based on reported standardization studies.[7a,8] Gene ontology analysis of initial microarray data

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suggested that most of the highly expressed genes among the top most **K**-induced genes are associated with gamete generation (Supporting Information, Figure S1). Further studies were carried out using purified **K** (Figure S2) and the control compounds **O** and **K-OMe** (Figure 1). HDFs were treated with the effectors at optimal conditions, at a final concentration of 1 μM for 48 h.

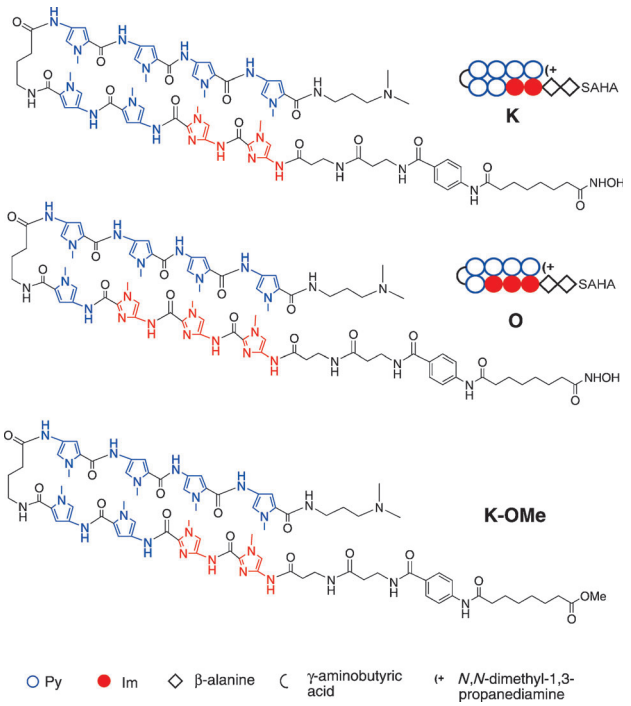

Figure 1. Chemical structures of SAHA-PIP **K** and the controls **O** and **K-OMe**. Im = imidazole, Py = pyrrole.

Effects of cytotoxicity on the changes in gene expression were precluded, as the effectors (**K**, **O**, and **K-OMe**) were not toxic to HDF even at a concentration of 10 μM (Figure S3). Using the microarray data, we generated a heat map of the top 200 up- and down-regulated genes in cells treated with **O** and **K**, which suggested unique transcriptional activation (Figure S4). Functional analysis of **K**-induced genes (> 5-fold) using ingenuity pathway analysis (IPA) substantiated that **K** alone could activate several key genes that confer to gametogenesis (Tables S1 and S2). In contrast, **O**-induced genes belonged to a different category of biological processes (Tables S3 and S4). Likewise, SAHA-induced genes also conferred to another set of biological processes (Tables S5 and S6). The heat map of the summarized expression profile of the gametogenesis-associated genes suggested that these usually conserved genes are overexpressed only in **K**-treated HDFs and not in SAHA-, **O**-, or DMSO-treated HDFs (Figure 2a). Pathway analysis of the **K**-induced genes in HDFs suggested a potential direct interaction between *MOV10L1*, *PIWIL1*, and *PIWIL4* and other factors that connect *TDRD1* and *PIWIL1* (Figure 2b). In line with this microarray data, **K** induced a significant, approximately 500-fold enhancement of endogenous expression of both *MOV10L1* and *PIWIL1* in HDFs (Figure 2c and d). **K** also induced approximately 50-, 150-, 12-, and 5-fold expression enhancements of *PIWIL2*, *PIWIL4*, *TDRD1*, and *TDRD9*, respectively (■, Figure 2e–h). Interestingly, **K** also activated *DAZL* and *RBMY*, which are the key gametogenesis genes (■, Figure 2i,j). The importance of the activity of both PIP and HDAC was further substantiated, as none of these gametogenesis-related genes could be up-regulated by SAHA and **K-OMe. O**, which entails one extra imidazole compared to **K**, could not induce any germ cell genes, which suggests site-specific bioactivity (■, Figure 2c–j). Thus, **K** concomitantly modulates the intricate germ-cell-associated gene network by triggering the endogenous expression of PIWI pathway genes in only 48 h.

We performed chromatin immunoprecipitation (ChIP) analysis to evaluate the acetylation status of the PIWI-pathway-related genes in effector-treated HDFs. Only in HDFs treated with **K**, the acetylation was notably enriched in the promoter region of the PIWI-pathway-associated factors (*MOV10L1*, *PIWIL1*, *PIWIL2*, *PIWIL4*, *TDRD1*, *TDRD9*; ■, Figure 3a–f). Interestingly, *MOV10L1*, which had been induced by more than 500-fold, experienced an approximately 30-fold enrichment in hyperacetylation in **K**-treated HDFs (Figures 2c and 3a). Instead, a mild enrichment in acetylation could be observed in *TDRD9* (induced by about 5-fold; Figures 2h and 3f).

Thus, a correlation was observed between acetylation and the gene expression pattern. The absence of an enrichment in the acetylation level after treatment with **K-OMe**, **O**, or SAHA (Figure 3a–f) suggests that **K** alone induces transcriptionally permissive chromatin in the promoter region of PIWI pathway genes. We then evaluated the effect of **K** on the acetylation of histone H3 by mapping the global occupancy of H3Ac through ChIP-Seq analysis. Based on microarray, gene expression, acetylation, and pathway analysis, **K** displays a relatively potent effect on *PIWIL1*. Consistently, *PIWIL1* showed an increased H3Ac occupancy in **K**-treated HDFs when compared with that in DMSO-treated cells (Figure 3g). Motif analysis indicated that about 39% of the motifs could be identified with a **K** binding site (Table S7).

As an imbalance of PIWI-associated gene expression could lead to unsuccessful germ cell development, **K** could be developed for versatile applications.[12b,15] Interestingly, some of the **K**-induced genes are known to associate with each other. *MOV10L1* associates with PIWI proteins to regulate meiosis by the piRNA pathway that is associated with spermatogenesis, and the expression pattern of *Mov10l1* is comparable to that of *Piwil2*.[16] Tudor family proteins control the piRNA pathway, and *Tdrd1* acts as a molecular scaffold for PIWI proteins.[17,18] In the germ lines of male mice, the two TDRD members TDRD9 and TDRD1 cooperate in the PIWI pathway to serve as the essential component of the male germ line by forming TDRD9–PIWIL4 and TDRD1–PIWIL2 complexes, respectively.[19] As **K** induces *MOV10L1* along with the tudor family genes, targeted transcriptional activation of the core PIWI pathway genes and subsequent activation of some germ cell markers could be inferred as the probable mechanism behind the **K** effect. **K** also activated factors related to spermatogenesis that is associated with the JAK-STAT signaling pathway (Figure S1).

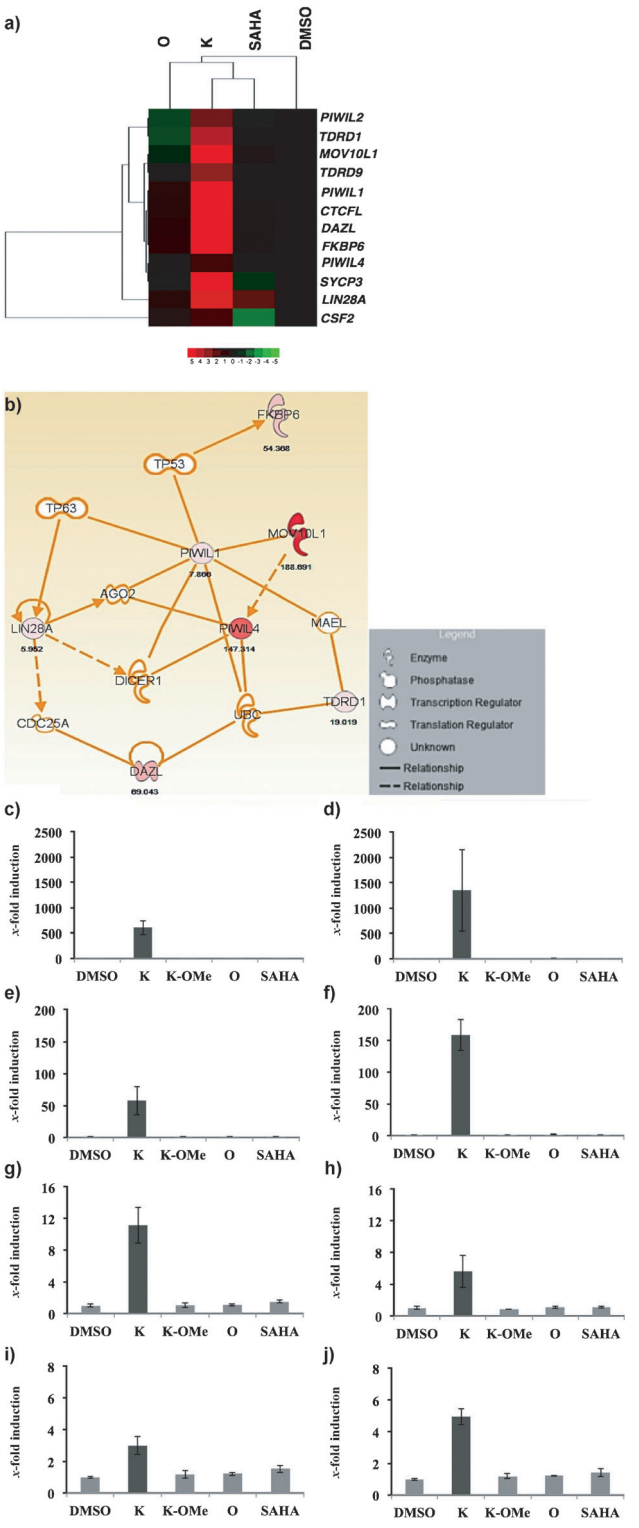

Figure 2. a) Heat map based on unsupervised hierarchical clustering analysis of germ cell gene expression data from fibroblasts treated with DMSO (set as the control), SAHA, SAHA-PIP **K**, and **O**. Each result is a combination of data from three plates. b) Path explorer analysis of gametogenesis-associated genes that are regulated in **K**-treated HDF. Color strength signifies the level of up-regulation. The white node represents the factor that connects the input list. Quantitative real-time (qRT)-PCR analysis of c) *MOV10L1*, d) *PIWIL 1*, e) *PIWIL 2*, f) *PIWIL 4*, g) *TDRD 1*, h) *TDRD 9*, i) *DAZL*, and j) *RBMY1B*. Expression profile of a HDF treated with **K** (1 μM) for 48 h (■); expression profiles of HDFs treated with SAHA, **O**, and **K-OMe** under the same conditions (■). Cells treated with DMSO (0.1%) were taken as an internal standard. Each bar represents the mean value ± SD from 24 wells.

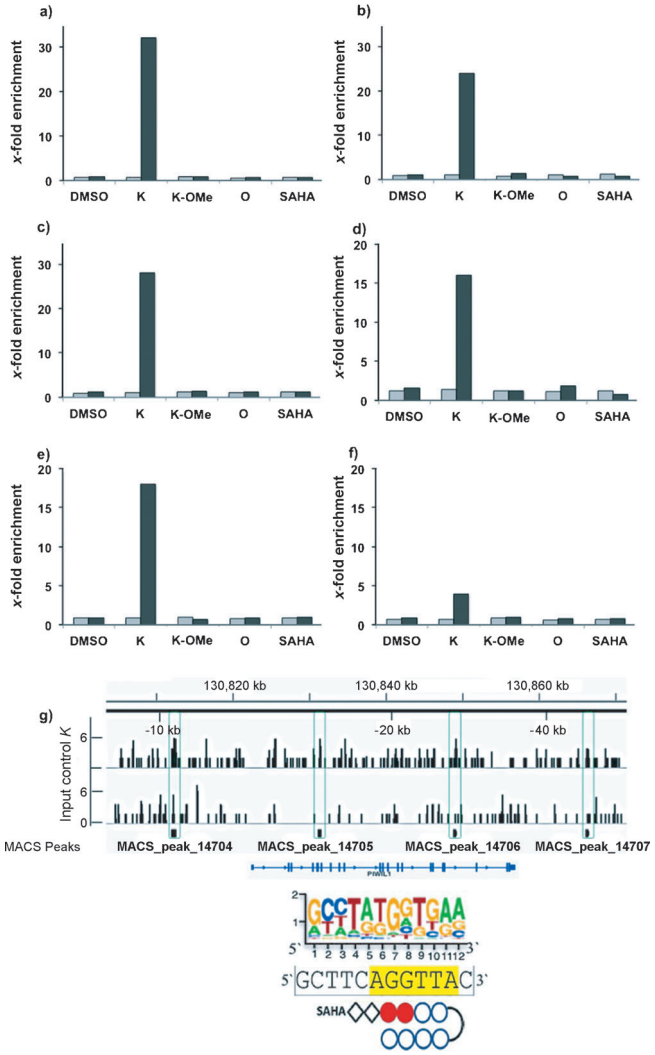

Figure 3. Treatment of HDF cells with effectors as described in Figure 2. After immunoprecipitation with the H3Ac antibody, the amount of promoter sequence of a) *MOV10L1*, b) *TDRD 1*, c) *PIWIL 1*, d) *PIWIL 2*, e) *PIWIL 4*, and f) *TDRD 9* was determined by quantitative PCR. The increase in acetylation enrichment was calculated by normalizing the data against the input DNA and by normalizing the enrichment with the IgG antibody. Each bar represents the average value of 24 wells. ■: IgG; ■: H3Ac. g) ChIP-Seq analysis. Peak calling by MACS 1.4.2 suggested enriched motifs in the *PIWIL 1* region that may result in the enhancement of the acetylation by **K**.

As chromatin remodeling is not an insulated event, transcriptional activation may also involve other epi-enzymes, as shown for a mouse fibroblast.[7a] Thus, the development of **K** may uncover a common unknown epigenetic association that exists among these fundamental germ cell genes. **K** targets a sequences of only six base pairs; however, recognition of 15–16 base pairs is necessary to target a single site within the

human genome, which consists of three billion base pairs. Nevertheless, PIPs, such as those targeting a sequence of 16 base pairs,[20] could be developed to regulate specific germ-cell-associated gene(s) and aid us to understand the actual mechanism of the **K** effect.

Histone modifications are orchestrated by a suppositional histone code, which is believed to be the key to their development.[21] In this regard, site-specific histone-modifying SAHA-PIPs suggest exciting opportunities in cellular reprogramming to activate the usually conserved developmental genes in a somatic cell. Unlike other programmable transcriptional activators, PIPs could bind with methylated sequences and have demonstrated superior specificity than natural binding proteins.[22] A recent report showed that modification of SAHA in the structure of SAHA-PIP could shift their specificity to different HDAC enzymes.[23] Therefore, it is possible to conjugate different chromatin modifiers and/or signaling pathway inhibitors for versatile applications.[1,24] Multifunctional small molecules such as SAHA-PIPs may precisely orchestrate the intricate transcriptional machinery that dictates cell fate. However, several factors, including cell permeability, accessibility, and stochastic epigenetic modifications, need to be addressed. Our strategy to employ programmable small molecules could also be developed for the reprogramming of cells from fibroblasts to a germ-cell-like state.

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