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Effects of Epigenetic Drugs (Vorinostat, Decitabine) on Metabolism-Related Pathway Factors in Leukemic Cells

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Abstract: Novel tools for data-evaluation from gene-expression arrays allow analyses of biochemical pathways which may be influenced by treatment with epigenetic drugs which have originally been designed for re-activation of so-called tumour-suppressor genes that are known as key-molecules regulating differentiation or cell death in malignancy. Considering the fact that these processes are tightly associated with energy metabolism, this study evaluated the expression signature of prominently regulated pathways in the KG-1-leukemia cell line: Following a 3-day incubation, effects of pharmacologic concentrations from the histone-deacetylase inhibitor SAHA (suberoyl anilide hydroxamic acid, vorinostat) and the methylation-inhibitor desoxy-azacytidine (DAC) were comparatively analysed by transcriptional profiling (based on Affymetrix Human GeneChip Gene 1.0 ST microarrays) and quantitative real time PCR. Expression factors for pathways were calculated for comparative analyses. Epigenetic drugs SAHA and DAC had a downregulatory effect on metabolic pathway factors of carbohydrate metabolism and mitochondrial beta oxidation. Our data confirm SAHA-mediated downregulation of the histone deacetylase SIRT which regulates AKT-phosphatase. Associated pathways lead to regulation of numerous genes, including an upregulation of FOXO transcription factors. These regulatory networks are known for their crucial role in stem cell homeostasis and provide a mechanistic explanation for the fact that the number of SAHA-targeted genes (1392 up, 2651 down) exceeds the number of DAC-targeted genes (60 up, 15 down), besides known effects on cell-cycle-arrest and apoptosis induced by both drugs.

Thus, our data underline that epigenetic mechanisms are tightly associated with malignancy-associated metabolic control at least at 3 levels, starting from (i) glucose-uptake over (ii) mitochondrial pathways to (iii) AKT-PTEN-FOXO-signalling. All of them are known to be regulated by caloric restriction. We propose that these interactions should be carefully considered in clinical application, providing the basis for optimization of drug-combinations and complex treatment strategies.

Keywords: Epigenetics, vorinostat, decitabine, energy metabolism, KG1-cells.

1. INTRODUCTION

It is generally accepted that epigenetic active drugs such as aza-nucleosides and histone-deacetylase-inhibitors act *via* re-activation of a large panel of genes including so-called tumor suppressor genes responsible for cell cycle arrest, differentiation and apoptosis in hematologic malignancies [1, 2]. The drugs have been well characterized as inhibitors of DNMTs (DNA methyl transferase) and are known since more than 20 years (e.g. [3-7]). Their application is promising because of their low toxicity to normal cells and their specific biological effects on a large number of cellular processes.

These drugs should actually target epigenetic processes like histone modifications (e.g. acetylation) and/or DNA-CpG methylation. However, recent studies using high sensitive microarray platforms for comparative evaluation of gene-methylation- and respective mRNA-expression-patterns indicate that, also in responders, these epigenetic drugs do not induce significant changes in gene expression driven by changes of the epigenetic DNA methylation status [8, 9], and therefore, the exact therapeutic molecular targets of epigenetic drugs still remain to be clarified.

It appears possible that aza-nucleosides and histone-deacetylase-inhibitors also target epigenetic processes which are tightly associated with malignancy-associated features of energy metabolism [10]. The rationale for this is based on the fact that increased supply of energy from carbohydrate- and fat catabolism confers a significant growth advantage to leukemias and cancer. An increased glycolytic activity of leukemia- and cancer cells produces an acidic environment

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which is more toxic to the adjacent tissue compared with malignant cells themselves [11].

Enhanced energy metabolism in malignancy is closely associated with a changed availability of acyl groups from fatty acid catabolism. As fatty acids represent a source of acyl groups which are substrates both for cytoplasmic and nuclear acyl- (or acetyl-) coenzyme A, the latter play a role for histone acetylation and thus gene activation / regulation. Histone-deacetylases which may be stimulated in response to a reduced availablity of intranuclear acyl groups, induce a hypoacetylation of histones in malignancy and promote a tighter packing of chromatin and thus inhibit transcription of critical genes such as so-called tumor suppressors which is also associated with promoter-methylation of these genes. Recent data showed that histone-deacetylase (HDAC) - inhibitors of distinct chemotypes have the potential to reverse this by downregulating glucose transporter 1 (SLC2A1) - mediated glucose transport and inhibition of hexokinase (HK1) – enzymatic activity [12]. In addition, several DNA-demethylating agents such as DAC exert this activity by promoting apoptosis and growth arrest at different direct or indirect pathways which may also regulate (de)differentiation of leukemic cells.

Array technologies have turned out as powerful tools for evaluating biological basal processes such as metabolism where reliability has been confirmed by comparative analyses of proteomic and transcriptomic arrays [13]. Comparative analyses of methylation-specific DNA-arrays with gene-expression arrays showed that elements of the forkhead box of transcription factors are hypermethylated and transcriptionally downregulated together with an aberrant upregulation of metabolic genes in KG-1 cells [14] referring to array GSM73642 in the GEO dataset. As downregulation of these so-called FOXO genes plays a key role in aberrant upregulation of carbohydrate metabolism in malignancy [15], the aim of this study was to find out, if re-activation of these and other genes by epigenetic drugs might induce a downregulation of metabolic pathways to a level known for normal (stem) cells.

This study applies novel analytic tools for exploration of biochemical pathways [16-18] from gene expression arrays to visualize expression patterns from metabolic pathways in the KG-1 cell line treated with known epigenetic drugs SAHA and DAC.

2. MATERIALS AND METHODS

2.1. Cell Culture

KG-1 cells (obtained from the American Type Culture Collection, ATCC, Manassas, VA) were seeded in petri dishes (8 cm in diameter) or culture flasks in RPMI 1640 supplemented with 5% glutamine, 10% FCS, 100 units/mL penicillin G sodium and 100 µg/ml streptomycin sulphate at a concentration of 300.000 cells/ml medium. Cells were incubated with either 0.5 µM SAHA or 5 µM DAC for 72h in comparison to untreated controls.

2.2. RNA-Isolation and Definition of Gene-Expression-Profiles

Isolated RNA (using eg the PROMEGA Z-3105 RNA-Isolation system) was used to determine, which genes and associated regulative pathways are affected by treatments with specific drugs including SAHA or desoxy-azacytidine (DAC).

Analysis and data evaluation for such Affymetrix Arrays (Type Human Gene 1.0 ST Array) was commercially obtained from an internationally certified institution (Kompetenzzentrum für Biofluoreszenz, Regensburg). "Pathvisio" [16, 18] and "iPath" [17] software packages were applied for specific analyses of defined pathways from Affymetrix Arrays (Type Human Gene 1.0 ST Array).

2.3. Calculation of Expression Factor for a Pathway (PEF)

To get a factor, which describes the regulation of a pathway in a qualitative manner, we adapted published algorithms [19, 20] for defining the following formula:

$$\mathrm{PEF} = \frac{\Sigma \log_2 signals^{Treat} - \Sigma \log_2 signals^{Contr}}{n^{\text{involved Genes}}}$$

Log_2 signals represent the intensity of gene-expression on the Human Gene 1.0 ST Array: Low expression levels are represented by log_2signals <5, median basic expression levels have log_2signals >5, and high expression levels refer to log_2signals. Basic expression levels (BELs) are shown in Suppl. Figs. (**1-3**). Multiplication of BELs with PEF indicates the effect of each treatment on gene expression.

2.4. Gene Expression Analysis by qRT-PCR (Quantitative Reverse Transcriptase PCR)

For comparative analyses cDNA was synthesized from 0.1 µg RNA using the 1st Strand cDNA Synthesis Kit as described by the supplier (Roche and/or Promega). The obtained cDNA was subjected to PCR amplification with a real-time cycler (Corbett Research). TaqMan gene expression probes & primers-sets (all from Applied Biosystems) in the respective master mix were used for amplification according to the suppliers suggested conditions. For normalization of expression we used the GAPDH TaqMan primer & probe-set in the same reaction vial (4310884E, Applied Biosystems). Relative quantification of mRNA within the samples was examined using the comparative Ct method:

($\Delta Ct_{untreated\ control} - \Delta Ct_{treated\ cells} = \Delta\Delta Ct$; relative quantity = $2^{-\Delta\Delta Ct}$) according to Livak & Schmittgen [21].

3. RESULTS

3.1. Epigenetic Drugs Downregulate Cell Proliferation

Drug modulated inhibition of cell multiplication was used to find an optimal concentration for drug treatment. For this purpose, cells were seeded and cultured for 72 hours with increasing concentrations of either SAHA or DAC. Fig. (**1**) demonstrates a dose dependent attenuation of cell multiplication. 0.5 µM and 5 µM, respectively, reduced the proliferation rate to about 40% and were, therefore, choosen for experiments.

3.2. Epigenetic Drugs Downregulate Key Pathways of Energy Metabolism

As shown in Tables **1-3**, pathways which are associated with cancer-related metabolic dysregulations were targeted

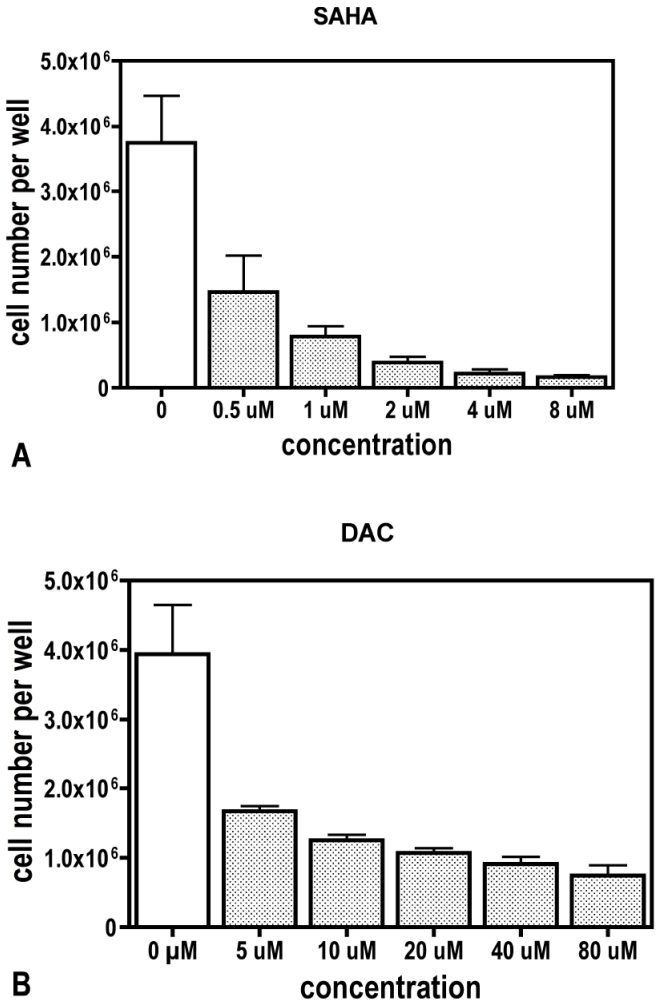

Fig. (1). SAHA (**A**) as well as DAC (**B**) downregulated cell multiplication of KG-1 cells. KG-1 cells were seeded at a density of 300,000 cells/ml and cultured for 1, 2 and 3 days with the indicated concentrations. Thereafter cells were counted with a cell counter (Cazy, Germany). Bars represents mean ± SD (cells/ml); *$P < 0.05$; n=4.

by the epigenetic drugs. As a consequence of drug-induced inhibition of glucose-uptake [12], genes regulating catabolism in both cytoplasm as well as in the mitochondria were down-regulated more efficiently by SAHA compared to DAC. Table **1** and Suppl. Fig. (**1**) demonstrate that of genes involved in glycolysis, 15 of 16 genes whose signal was strong enough to suggest a significant rate of mRNA expression, were down-regulated by SAHA; thereof 12 genes were strictly down-regulated (more than 2-fold), 3 weak (between 1.1-fold and 2-fold) and one was up-regulated. DAC regulated the expression similarily but at the used concentration it was less effective (Table **1**, Suppl. Fig. **1**). Catabolism is continued in the mitochondria by the enzymes of the "three carbonic acid" (TCA) cycle. In this pathway, 20 of 30 genes responsible for the catabolism of Acetyl-CoA were strongly down-regulated to less than 20% by SAHA, 3 weak or not regulated, and only 6 were up-regulated. Not surprisingly, out of the 6 up-regulated genes, three genes (PDK1, 2, 3) were inhibitors of enzymes catalysing the first step of this cycle (Table **2** and Suppl. Fig. **2**). Again, DAC had a weaker effect on gene regulation of those genes that led to the fact that less genes were regulated (19/30). Another pathway which is localized in the mitochondria is the "fatty acid beta oxidation" (FABO), for which 18 genes are suggested (Table **3**, Suppl. Fig. **3**, [16, 18]). Twelve genes were strongly down-regulated by SAHA while 5 were weakly attenuated and one was up-regulated. Similiarily as found with the other pathways DAC had a weaker effect on the down-regulation (Table **3** and Suppl. Fig. **3**). Evaluation of gene array results with qRT-PCR as shown in Fig. (**2**) analyzing expression of CPT1B by qRT-PCR yielded a comparable result.

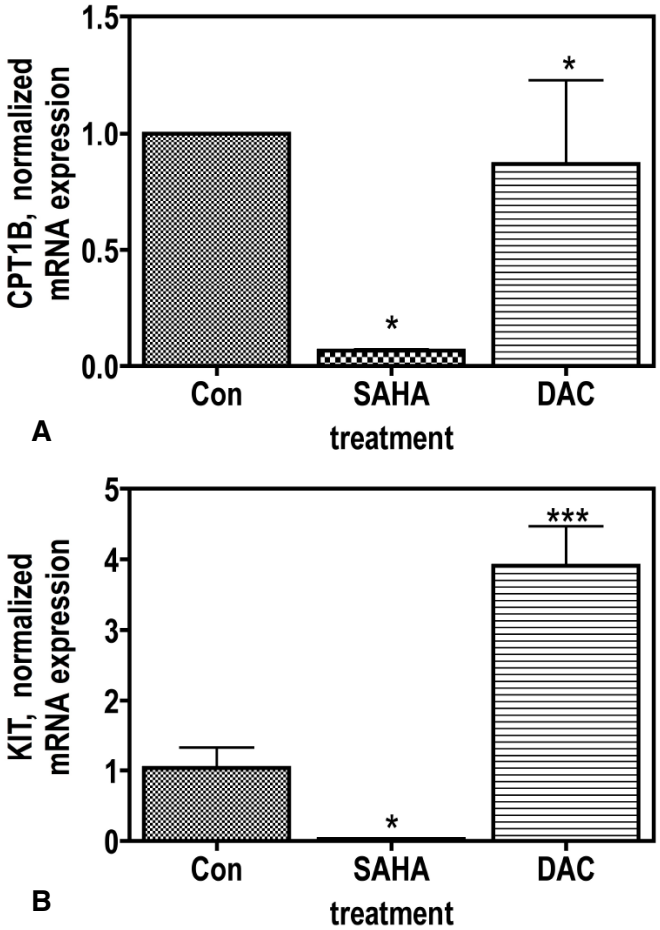

Fig. (2). CPT1B (**A**) and KIT (**B**) mRNA expression in SAHA and DAC treated KG-1 cells. KG-1 cells were treated for 72 hours with the 0.5 µM SAHA and 5 µM DAC. Thereafter, mRNA was isolated, reverse transcribed, and subjected to qRT-PCR. SAHA downregulated CPT1B as well as KIT mRNA expression. While DAC weakly downregulated CPT1B while it stimulated KIT mRNA expression significantly. The specific gene expression was normalized to 18S RNA expression. The bars represent mean ± SD. *$P < 0.05$; ***$P \leq 0.001$; $n = 3$.

Possibly as a kind of "rescue mechanism" initiated from deprival of energy from glycolysis, the key enzyme of gluconeogenesis, fructose-1,6-bisphosphatase 1 and 2 (FBP1 and FBP2), which is also responsible for prevention of metabolic acidosis during conditions of starving, was stimulated with SAHA and DAC.

Considering glycolysis most of the genes were down regulated by SAHA and a comparable effect was found with

Fig. (3). Venn-diagrams of genome wide gene expression analysis of untreated, DAC and SAHA treated KG-1 cells. (**A**) Blue and green coloured ellipsoids demonstrate downregulated genes by SAHA and DAC, respectively, relative to untreated controls. The overlaps refer to genes downregulated by both drugs. (**B**) Red and yellow coloured ellipsoids demonstrate upregulated genes by SAHA and DAC, respectively, relative to untreated controls. The overlaps refer to genes upregulated by both drugs.

Table 1. Targeted Genes of Glycolysis

Gene Code	Full name of the Gene	Regulation by SAHA	Regulation by DAC
ALDOA	Aldolase A. fructose-bisphosphate	0.35	0.93
GOT1	Glutamic-oxaloacetic transaminase 1	0.31	0.90
ENO1	Homo sapiens enolase 1. (alpha)	0.41	0.84
ENO2	Homo sapiens enolase 2 (gamma. neuronal)	0.70	1.03
GCDH	Glutaryl-Coenzyme A dehydrogenase	0.45	0.81
LDHA	Lactate dehydrogenase A	0.37	0.88
LDHB	Lactate dehydrogenase B	0.28	0.99
PFKL	Phosphofructokinase. liver	0.30	0.84
PFKP	Phosphofructokinase. platelet	0.36	0.79
PGAM1	Phosphoglycerate mutase 1	0.41	0.81
PGAM2	Phosphoglycerate mutase 2	1.77	1.12
PGAM4	Phosphoglycerate mutase family member 4	0.42	0.83
PGK1	Phosphoglycerate kinase 1	0.55	0.86
PKM2	Pyruvate kinase. muscle	0.82	0.92
SLC2A1	Solute carrier family 2 (GLUT1)	0,28	0,76
TPI1	Triosephosphate isomerase 1	0.22	0.80

Down regulated genes were, dependent or their level colored in green, up regulated in red. At the chosen concentration most of the genes were down regulated by SAHA. A comparable effect was found with DAC, although, the drug was less effective. The calculated expression factors for the pathways (PEF) were -1.21 for SAHA and -0.19 for DAC (see material and methods and Suppl. Fig. **1**)

DAC, although, the drug was again less effective. The calculated expression factors for the pathways (PEF) were -1.21 for SAHA and -0.19 for DAC (Table **1** and Suppl. Fig. **1**). This was also evident for the TCA circle where PEFs were -1.20 for SAHA and -0.17 for DAC (Table **2** and Suppl. Fig. **2**) and for mitochondrial beta oxidation were PEFs were -1.15 for SAHA and -0.23 for DAC (Table **3** and Suppl. Fig. **3**).

3.3. Epigenetic Drugs Target KIT- and FOXO-Associated Pathways in KG-1 Cells

As demonstrated in Table **4a**, oncogenic KIT signalling is exclusively suppressed by SAHA as indicated by downregulation of KIT (confirmed by qPCR as seen in Fig. **2**) and stimulation of its inhibitor PTEN, thus confirming this

Table 2. Targeted Genes of "Three Carbonic Acid" Cycle

Gene Code	Full Name of the Gene	Regulation by SAHA	Regulation by DAC
ACO2	Aconitase 2	0.32	0.78
CS	Citrate synthase	0.57	0.86
DLAT	Dihydrolipoamide S-acetyltransferase MITO?	0.31	0.83
DLD	Dihydrolipoamide dehydrogenase MITO?	0.32	0.89
DLST	Dihydrolipoamide S-succinyltransferase mito?	0.92	0.98
FH	Fumarate hydratase	0.36	0.77
IDH2	Isocitrate dehydrogenase 2	0.27	0.86
IDH3A	Isocitrate dehydrogenase 3	0.44	0.91
IDH3B	Isocitrate dehydrogenase 3 transcript variant 3	0.19	0.87
IDH3G	Isocitrate dehydrogenase 3 transcript variant 2	0.35	0.81
MDH1	Malate dehydrogenase 1	0.33	0.91
MDH2	Malate dehydrogenase 2	0.29	0.78
OGDH	Oxoglutarate (alpha-ketoglutarate) dehydrogenase	0.59	0.87
PC	Pyruvate carboxylase	0.42	0.62
PDHA1	Pyruvate dehydrogenase (lipoamide) alpha 1	0.47	0.83
PDHB	Pyruvate dehydrogenase (lipoamide) beta	0.26	0.89
PDK1	Pyruvate dehydrogenase kinase. isozyme 1	1.28	0.82
PDK2	Pyruvate dehydrogenase kinase. isozyme 2 mito?	1.18	0.97
PDK3	Pyruvate dehydrogenase kinase. isozyme 3mito?	1.20	0.83
PPM2C	Protein phosphatase 2C	1.14	1.13
PDP2	Pyruvate dehydrogenase phosphatase isoenzyme 2	1.50	0.91
SDHA	Succinate dehydrogenase complex. subunit A	0.34	0.83
SDHB	Succinate dehydrogenase complex. subunit B	0.16	0.81
SDHC	Cytochrome b large subunit of complex II	1.03	1.01
SDHC	Succinate dehydrogenase complex. subunit C	1.33	0.77
SDHD	Succinate dehydrogenase complex. subunit D	0.23	0.98
SDHD	Succinate dehydrogenase complex. subunit D	0.40	0.82
SUCLA2	Succinate-CoA ligase. ADP-forming. beta subunit	0.28	0.97
SUCLG1	Succinate-CoA ligase. alpha subunit mito?	0.39	0.88
SUCLG2	Succinate-CoA ligase. GDP-forming. beta subunit mito?	0.35	0.69

At the chosen concentration most of the genes were down regulated by SAHA. A comparable effect was found with DAC, although, the drug was less effective. The calculated expression factors for the pathways (PEF) were -1.20 for SAHA and -0.17 for DAC (see material and methods and Suppl. Fig. **2**).

specific feature of HDAC-inihibitors [22]. The serine /threonine kinase AKT1 regulates both KIT and the family of forkhead transcription factors (FOXO1, 3, 4, Table **4b**). The strong stimulation of FOXO resulting from epigenetic downregulation of SIRT1, was also exclusively observable by SAHA [23-25].

3.4. Comparative Analyses Explain Common Regulatory Mechanisms of SAHA and DAC

Venn diagrams (Fig. **3**) showed that the majority of significantly (= more than 2-fold) regulated mRNAs were targeted by SAHA: Downregulation (Fig. **3A**) was observed in 2651 (pseudo)genes with SAHA as compared to 15 with

Table 3. Targeted Genes of Fatty Acids ß-Oxidation

Gene Code	Full Name of the Gene	Regulation by SAHA	Regulation by DAC
ACADM	Acyl-Coenzyme A dehydrogenase. C-4 to C-12 straight chain	0 31	1.00
ACADS	Acyl-Coenzyme A dehydrogenase. C-2 to C-3 short chain	0.70	0.86
ACADVL	Acyl-Coenzyme A dehydrogenase. very long chain	0.53	0.81
ACAT1	Acetyl-Coenzyme A acetyltransferase 1	0.22	0.83
ACSS1	Acyl-CoA synthetase short-chain family member 1	0.64	0.85
ACSS2	Acyl-CoA synthetase short-chain family member 2	0.40	0.67
CPT1A	Carnitine palmitoyltransferase 1A (liver)	1.25	0.94
CPT1B	Carnitine palmitoyltransferase 1B (muscle)	0.65	0.94
CRAT	Carnitine acetyltransferase (CRAT)	0.60	0.83
DCI	Dodecenoyl-Coenzyme A delta isomerase	0.47	0.94
DLD	Dihydrolipoamide dehydrogenase	0.32	0.89
DECR1	Homo sapiens 2.4-dienoyl CoA reductase 1	0.38	0.91
ECHS1	Enoyl Coenzyme A hydratase. short chain. 1	0.28	0.83
GCDH	Glutaryl-Coenzyme A dehydrogenase	0.45	0.81
HADH	Hydroxyacyl-Coenzyme A dehydrogenase	0.21	0.83
HADHA	3-ketoacyl-Coenzyme A thiolase. alpha subunit	0.42	0.79
HADHB	3-ketoacyl-Coenzyme A thiolase. beta subunit	0.42	0.88
SLC25A20	Homo sapiens solute carrier family 25	0.47	0.80

Down regulated genes were, dependent or their regulation colored in green, up regulated in red. At the chosen concentration most of the genes were down regulated by SAHA. A comparable effect was found with DAC, although, the drug was less effective. The calculated expression factors for the pathway (PEF) were -1.15 for SAHA and -0.23 for DAC (see material and methods and Suppl. Fig. **3**)

DAC. Upregulation (Fig. **3B**) was evidenced for 1392 mRNAs with SAHA, but only 60 with DAC.

Considering downregulated genes, there are remarkable common features between DAC and SAHA as shown by the fact that 5/15 DAC-downregulated (pseudo)genes were also downregulated by SAHA (Fig. **3A**) and 37/60 DAC-upregulated (pseudo)genes were also upregulated by SAHA. However, as all of them belong to the large group of small nuclear and nucleolar RNAs, a functional interpretation of this observation awaits to be delineated.

4. DISCUSSION

Results of this study show that chromatin-targeting drugs (DAC and SAHA) have the potential to target key metabolic pathways which are tightly associated with malignancy (Tables **1-3** and Suppl. Figs. **1-3**). Our data underline that SAHA targets malignancy-associated metabolic control at least at 3 levels, starting from (i) glucose-uptake over (ii) mitochondrial pathways to (iii) AKT-PTEN-FOXO-signaling. DAC-mediated targeting of mitochondrial pathways appears to be related to its apoptosis-promoting activity [26] and a slight stimulation of forkhead transcription factors by DAC may be promoted by demethylation of these hypermethylated genes [14].

Considering the crucial role of targeting these pathways in treatment of cancers and leukemias [11, 12, 27], this may provide an explanation for the clinical efficiency of those drugs, and may indicate, besides their known effects on epigenetic re-activation of (so-called tumor suppressor) genes involved in regulation of cell cycle and apoptosis [3, 4], the systemic consequences of epigenetic regulations.

The observed downregulation of energy metabolism may also be related to inhibition of cytoplasmic genes encoding mitochondrial proteins, as prominently observed with epigenetic drugs (Table **1** and Suppl. Figs. **1-3**). Recent reviews indicate that mitochondria also play a key role in treatment-resistance of many cancers, thus confirming their increasing attractiveness as targets in cancer-chemotherapy [27], in addition to their known role in energy metabolism [28]. Mitochondrial energy genes are dispersed across the chromosomes plus the maternally inherited mitochondrial DNA. Moreover, the cells and tissues most affected by aging and malignancy are those most reliant on mitochondrial energy. The mitochondrion lies at the interface between

Table 4a. Targeting of Important Genes of Hematopoietic Pathways AKT1, KIT, and PTEN

Gene Code	Full Name of the Gene	Regulation by SAHA	Regulation by DAC
AKT1	v-akt murine thymoma viral oncogene homolog 1	0,46	0,84
KIT	v-kit feline sarcoma viral oncogene homolog	0,50	1,16
PTEN	Phosphatase and tensin homolog	1,68	0,89

Down regulated genes were, dependent on their regulation, colored in green, up regulated in red. At the chosen concentration most of the genes were down regulated by SAHA. DAC differentially regulated those genes.

Table 4b. Expression of Genes of the FOXO and SIRT Transcription Factor Families

Gene Code	Full name of the Gene	Regulation by SAHA	Regulation by DAC
FOXO1	Forkhead box O1	2,20	1,09
FOXO3	Forkhead box O3 transcript variant 1	2,03	1,02
FOXO4	Forkhead box O4	3,06	1,07
SIRT1	Sirtuin 1	0,43	1,10
SIRT2	Sirtuin 2	0,75	0,98
SIRT5	Sirtuin 5	0,82	0,79
SIRT6	Sirtuin 6	0,97	1,05
SIRT7	Sirtuin 7	0,71	0,96

Down regulated genes were, dependent on their regulation, colored in green, up regulated in red. At the chosen concentration most of the genes were down regulated by SAHA. DAC except for SIRT5, didn't influence gene expression at the chosen concentration.

environmental calories and human physiology, as well as metabolic diseases. Both the signal transduction pathways and the epigenome are regulated by ATP mediated protein phosphorylation, acetylation *via* acetyl-CoA, and methylation by S-adenosylmethionine, all driven by mitochondrial high energy substrates modulated by available energy. Furthermore, mitochondrial redox chemistry regulates reactive oxygen production and thiol/disulfide chemistry and these also regulate cellular signaling and function.

Drug-induced downregulation of glycolysis was associated with stimulation of energy storage by gluconeogenesis (Table **1** and Suppl. Fig. **1**). Enhanced gluconeogenesis is a hallmark of aging eukaryotic cells [29], but may also be a "rescue-mechanism" in case of starvation. In mammals, a fasting-inducible switch consisting of the histone-acetyltransferase p300 (EP300) and the nutrient-sensing deacetylase sirtuin1 (SIRT1) which is also targeted by SAHA, is essential for maintenance of energy balance during fasting by activation of gluconeogenesis [25, 30].

From a therapeutic standpoint, the increasing knowledge on acetylation-dependent mechanisms of FOXO regulation expands the potential repertoire of pharmacological HDAC inhibitors [23, 31-33]. Yang *et al.* [25] showed that SIRT1 downregulates FOXO by deacetylation. Results of this study showed that three FOXO genes (FOXO1, FOXO3 and FOXO4 are significantly stimulated when SIRT1 was inhibited in KG-1 cells with SAHA (Table **4**).

Although, the present report appears to be the first study, showing the metabolic effects of epigenetically active drugs, downregulatory effects on carbohydrate metabolism have been expected both with SAHA and DAC as a consequence of induced mitochondrial damage [34-37]. In addition, both these pharmacologic compounds are also known for their antiproliferative effects resulting from cell cycle inhibition [3, 4].

The mechanism for the antiproliferative effect of SAHA which targets the majority of expressed mRNAs as seen in the VENN diagrams (Fig. **3**) is believed to be the result of inhibition of HDAC activity, resulting in the accumulation of acetylated proteins, including histones. Inhibition of HDAC activity by SAHA has multiple cellular effects. These effects include an alteration in the transcription of a finite number of genes (2–5% of expressed genes) *via* acetylation of histones and transcription factors, as well as effects such as cell cycle arrest *via* inhibition of mitosis. As an antimetabolite DAC also induces cell cycle arrest by stimulating cell cycle inhibitors as well as by inhibiting DNA-repair in the G2 phase of the cell cycle because of its structural similarity to desoxycytosine [26].

Taken together, epigenetic drugs (especially SAHA) have the potential to simulate a situation of caloric restriction by (i) inhibiting glucose uptake, which results in (ii) a downregulation of carbohydrate catabolism, which is further stimulated by mitochondrial damage as evidenced by a downregulated beta oxidation and (iii) a stimulation of FOXO transcription factors as a consequence of downregulated SIRT and AKT resulting from SAHA-mediated HDAC-inhibition and possibly also by demethylation of these hypermethylated forkhead genes [14]. In addition to these 3 effects on the transcriptome-level, there is a 4th effect on the proteome-level as shown by regulation of STAT (signal transducer and activator of transcription). Signaling by STAT1 requires HDAC-activity

[38]. Recent data indicate that STAT1- dependence of energy-metabolism links tumour growth and radioresistance to the Warburg effect [39].

Thus, our data confirm epigenetic effects on differentiation by re-activation of tumor-suppressors in leukemic cells [3, 4, 40-42]. SAHA and DAC are well established for therapy protocols in myelodysplastic syndromes and leukemias (e.g. [5, 43-45]). However, it is intriguing that additive or synergistic effects against tumor cell growth have been observed in most reported preclinical studies where SAHA was combined with chemotherapeutic agents or radiation [45, 46].

Since these compounds are coming to the fore as antineoplastic agents, the present findings raise the question of whether they could be pressed into service against the raising tide of metabolic diseases and the associated risks for malignancies [47, 48]. Furthermore, data presented in this study indicate that novel tools have the potential to illustrate the multitargeted effects of epigenetic drugs in treatment of malignancies including leukemias. Visualization of drug-effects on such complex processes requires a paradigm-change from „single-parameter-analyses“ to comparative evaluation of pathways, thus illustrating the innovative potential of the present study.

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SUPPLEMENTARY MATERIAL

This article also contains supplementary material and it can be viewed online along with the article at publisher’s website.

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