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HTP Nutraceutical Screening for Histone Deacetylase Inhibitors and Effects of HDACis on Tumor-suppressing miRNAs by Trichostatin A and Grapeseed (*Vitis vinifera*) in HeLa cells

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Abstract. *Background/Aim: Aggressive tumor malignancies are a consequence of delayed diagnosis, epigenetic/phenotype changes and chemo-radiation resistance. Histone deacetylases (HDACs) are a major epigenetic regulator of transcriptional repression, which are highly overexpressed in advanced malignancy. While original chemotherapy drugs were modeled after phytochemicals elucidated by botanical screenings, HDAC inhibitors (HDACi) such as apicidin, trichostatin A (TSA) and butyrate were discovered as products of fungus and microbes, in particular, gut microbiota. Therefore, a persistent question remains as to the inherent existence of HDACis in raw undigested dietary plant material. In this study, we conduct a high-throughput (HTP) screening of ~1,600 non-fermented commonly used nutraceuticals (spices, herbs, teas, vegetables, fruits, seeds, rinds etc.) at (<600 μg/ml) and food-based polyphenolics (<240 μg/ml) for evidence of HDAC activity inhibition in nuclear HeLa cell lysates. Materials and Methods: Human HDAC kinetic validation was performed using a standard fluorometric activity assay, followed by an enzymatic-linked immuno-captured ELISA. Both methods were verified using HDACi panel drugs: TSA, apicidin, suberohydroxamic acid, M344, CL-994, valproic acid and sodium phenylbutyrate. The HTP screening was then conducted, followed by a study comparing biological effects of HDACis in HeLa cells, including analysis of whole-transcriptome non-coding RNAs using Affymetrix miRNA 4.1-panel arrays. Results: The HTP screening results confirmed 44/1600 as potential HDACis to which 31 were further eliminated as false-positives. Methodological challenges/concerns are addressed regarding plant product false-positives that arise from the signal reduction of commercial lysine development reagents. Only 13 HDACis were found having an IC_{50} under <200 μg/ml: Grapeseed extract (Vitis vinifera), Great burnet root (Sanguisorba Officinalis), Babul (Acacia arabica), Chinese gallnut (Melaphis chinensis), Konaberry extract (Coffea arabica), Uva Ursi (Arctostaphylos uva ursi), Green tea (Camellia sinensis), Meadowsweet (Filipendula ulmaria), Sassafras (Sassafras officinale), Turkey rhubarb (Rheum palmatum), epigallocatechin gallate (EGCG), gossypol and gallic acid. Next, we investigate the biological consequence of HDACi panel drugs in HeLa cells, where the data suggest predominant effects are anti-mitotic rather than cytotoxic. Lastly, differential effects of TSA vs. GSE at sub-lethal concentrations tested on HeLa cells show 6,631 miRNAs expressed in resting cells, 35 significantly up-regulated (TSA) and 81 up-regulated (GSE), with several miRNAs overlapping in the upward direction by both GSE and TSA (e.g. hsa-miR-23b-5p, hsa-miR-27b-5p, hsa-miR-1180-3p, hsa-miR-6880-5p and hsa-mir-943). Using DIANA miRNA online tools, it was determined that GSE and TSA simultaneously cause overexpression of similar miRNAs predicted to destroy the following influential oncogenes: NFkB, NRAS, KRAS, HRAS, MYC, TGFBR1, E2F1, E2F2, BCL21, CDKN1A, CDK6, HIF1a, and VEGFA. Conclusion: The data from this study show that plant- based HDACis are relatively rare, and can elicit a similar pattern to TSA in up-regulating miRNAs involved with tumor suppression of HeLa cervical carcinoma.*

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Key Words: Screening, HDAC, grapeseed, miRNA, TSA, HeLa cells.

The term "epigenetics" encompasses a complex multi-component regulatory system which controls long term and transient changes in gene expression/silencing (1). Histone deacetylases (HDACs) are regulators of transcriptional repression where they catalytically cleave/remove acetyl groups from the epsilon lysine residues on histone tails, creating a net positive charge and greater affinity for negatively

charged phosphate groups on DNA. This mechanical interaction enables histone/nucleosome constriction and silencing of genes into compact-organized heterochromatin. As technology evolves, so does the classification of HDACS, which are known to include the following: zinc-dependent HDACs include HDACs1 (2, 3 and 8); Class II (4-7, 9, 10), Class IV (11) and nicotinamide regulated HDACs comprising Class III, Sirtuins.

Elevated expression of HDACs are evident in advanced malignancy, where inhibitors (HDACis) antagonize tumor growth (2, 3) antagonize tumor growth (2, 3), augment chemotherapy (4-7), reverse chemoresistance (3, 8, 9), attenuate metastasis, halt epithelial-mesenchymal transition (10-12) and block tumor immune evasion (13, 14). With the overwhelming surge of HDACis being sought worldwide for nearly every type of cancer (*e.g.* ovarian (15) neuroblastoma (16) pancreatic (17) liver (18) colon (19) and cervical cancer (20)), a database (HDACiDB) has been established to accommodate this wealth of information. (21) Several mechanisms have been discovered as to how HDACis carry out their anti-tumor effects, including a re-expression of tumor suppressors (*e.g.* p21, p27 BAX) (22, 23), down-regulation of oncogenes (*e.g.* TGF-beta1, MMP-1 and MMP-2 (24), survivin, beta-catenin, N-cadherin and vimentin) (25) and blocking tumor signaling processes (*e.g.* SMAD4, PI3K, PI3K/AKT/mTOR) (26-28). With advances in epigenetic technologies, many HDAC inhibitors have been documented to alter tumor suppressor miRNAs and thereby influence the growth of cancer (19, 29). Given that HDACis were first discovered as products of microorganisms including gut microflora from dietary fiber (30) and HDACi drug development continues to be modeled after microbial metabolites such as trans-cinnamic acid, FK228, YM753 (spiruchostatin A) (31-33), there remains a question as to if HDACis exist in undigested plant materials. In this study, we conduct a high throughput screening of ~1,600 commonly used nutraceuticals (plants, vegetables, fruits, herbs, vitamins *etc.*) and polyphenolics for the ability to inhibit HDAC enzymatic activity in HeLa nuclear lysates using a common fluorometric assay. We discuss methodological issues, confirm/validate HDACis using specific product linked ELISAs and then evaluate related epigenetic/biological consequences of two specific HDACis of diverse origin: trichostatin A (TSA) microbial *vs.* a plant based HDACi: grapeseed extract (GSE).

Materials and Methods

Hanks Balanced Salt Solution, (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES), ethanol, 96 well plates, general reagents and supplies, RNA extraction supplies were all purchased from Sigma-Aldrich (St. Louis, MO, USA) or VWR (Radnor, PA, USA). All microarray reagents and supplies were purchased from Affymetrix (Santa Clara, CA, USA) and natural products were purchased from Frontier Natural Products Co-op (Norway, IA, USA), Monterey Bay Spice Co (Watsonville, CA, USA), Mountain Rose Herbs (Eugene, OR, USA), Mayway Traditional Chinese Herbs (Oakland, CA, USA), Kalyx Natural Marketplace (Camden, NY, USA), a local organic fruit and vegetable market (New Leaf, Tallahassee, FL,USA), Florida Food Products Inc. (Eustis, FL,USA), Konaberry extract from Amazon Rainforest Inc, (Ledgewood NJ, USA) or Patel Brothers Indian Grocery (Tampa, FL, USA). Grapeseed Extracts were purchased from Hard Eight Nutrition LLC (Henderson, NV,USA), Futureceuticals (Momence, IL,USA), and from capsules obtained at General Nutrition Center (GNC) and Swanson Health Products (Fargo, ND, USA). HDAC activity assays were purchased from Abcam (Cambridge, MA, USA), Enzo Life Sciences through VWR (Radnor, PA, USA) and Epigentek (Farmingdale, NY, USA).

Cell culture. HeLa (ATCC® CCL-2™) cells were purchased from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in DMEM high glucose media [glucose 4,500 mg/L] containing 5% FBS, 4 mM L-glutamine, and penicillin/streptomycin (100 U/0.1 mg/ml). Culture conditions were maintained at 37˚C in 5% CO_2/atmosphere and every 2-3 days, the media was replaced and cells sub-cultured, and culture media was used for all *in vitro* experiments.

Herbal, compound and drug preparations. All natural chemicals and reference drugs were dissolved in dimethylsulfoxide (DMSO) [5-20 mg/mL], and crude herbs were prepared in absolute ethanol [50 mg/ml] after being diced, macerated and powdered, all stored at –20˚C. Serial dilutions were prepared in sterile HBSS + 5 mM HEPES, adjusted to a pH of 7.4 and solvent concentration of DMSO or absolute ethanol was maintained at less than 0.5% for all experiments.

Cell viability. Cell viability and cell counts were quantified using resazurin [7-Hydroxy-3H-phenoxazin-3-one 10-oxide] (Alamar Blue) indicator dye (34). A working solution of resazurin was prepared in sterile HBSS minus phenol red (0.5 mg/ml), and then added (15% v/v) to each sample. Samples were returned to the incubator for 2-4 h and reduction of the dye by viable cells (to resorufin, a fluorescent compound) was quantitatively analyzed using a Synergy HTX multi-mode reader (Bio-Tek, Winooski, VT, USA) with settings at [550/580], [excitation/emission]. For toxicity studies, HeLa cells were placed in growth media at a cell density of 0.5×10^5 cells/ml in 96 well plates and viability assessed at 24 h of incubation. For cell proliferation, HeLa cells were placed in growth media at a cell density of 0.04×10^5 cells/ml in 96-well plates and viability was assessed at 3-4 days incubation, using paclitaxel as a negative control.

HDAC activity. An HDAC activity assay [ab156064] Abcam (Cambridge, MA, USA) was used for high-throughput screening of natural products, being conducted in accordance with the manufacturers guidelines. A pre-read and subsequent post-read was acquired using a Synergy HTX Multi-Mode Reader Excitation: 355/40, Emission: 460/40, optics: Top, Gain: 37. Solvent controls were used to detect and account for product/developer signal decay and compared to the full enzyme reactant samples. Data was acquired using Gen5™ Data Analysis Software 2.06.10.

An ELISA based HDAC Activity /Inhibition direct assay # P-4035 (Epigentek Group Inc. Farmingdale, NY, USA) was used for dual detection validation. This product has major advantages in that

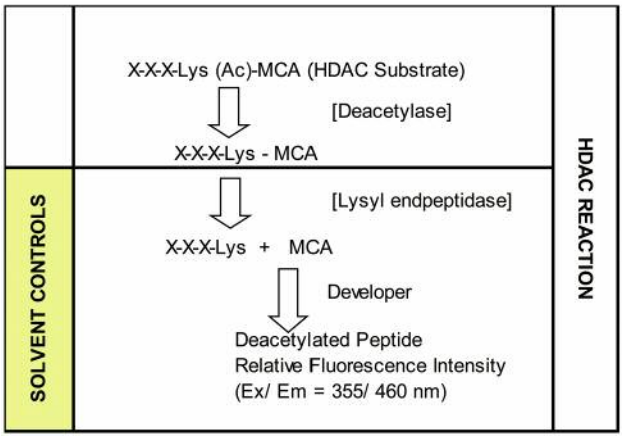

Figure 1. *HDAC activity assay layout. HDAC substrate is cleaved and then detected by a developer reaction solution containing lysyl endopeptidase. Determination of HDAC activity=Enzyme + TX+ Developer/Assay Solution – TX+ No Enzyme + Developer/Assay Solution [Solvent Control].*

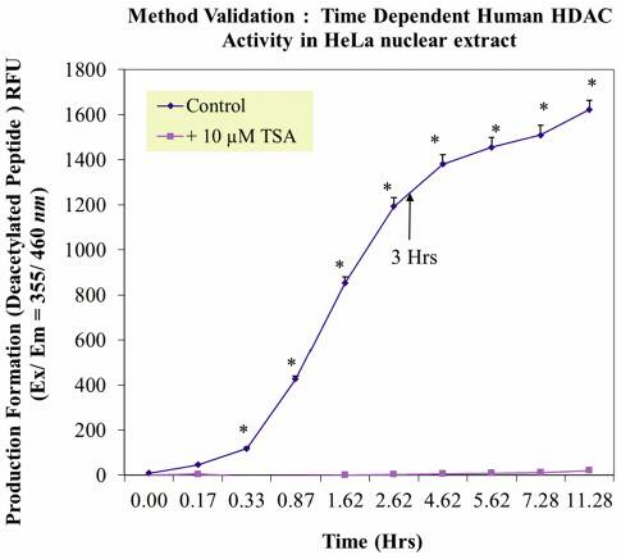

Figure 2. *Time-dependent HDAC enzyme activity in crude HeLa nuclear lysate at 37˚C±10 µM TSA (HDAC inhibitor). The data represent the formation of deacetylated peptide product quantities by relative fluorescent intensity (RFU) 355 (ex) /460 (em) presented as the mean±S.E.M, n=4. Time-dependent differences in the control were analyzed by a one-way ANOVA, followed by a Tukey post hoc test. *p<0.05. A two-way ANOVA between control and TSA inhibitor established significant differences between the groups *p<0.01.*

the substrate is bound to microplate wells, the enzyme converts the bound substrate to a product, and after several washes – the product is detected by ELISA. The assay was conducted according to the manufacturer's protocol, samples read on a Synergy HTX Multi-Mode Reader and data acquired using Gen5™ Data Analysis Software 2.06.10.

Genomic profiling of TSA vs. Grapeseed extract. miRNA profiling was accomplished using a FlashTag™ Biotin HSR RNA Labeling Kit on RNA spiked with oligo controls. Briefly, RNA was isolated, purified and quantified, spiked, diluted in ATP/Poly A Tail Master Mix and incubated a 37˚C heat block for 15 min. Flash Tag Biotin HSR ligation mix was added with T4 DNA ligase and incubated at RT for 30 min. The reaction was stopped, and a QC assay was run. The sample was hybridized to an Affymetrix array strip at 48˚C and added to a hybridization cocktail. Samples were denatured at 95˚C (thermocycler plates) for 5 min, followed by 45˚C for 5 min. Samples were analyzed using Affymetrix gene atlas software and analyzed by DIANA (35).

Docking and data analysis. Statistical analysis was performed using Graph Pad Prism (version 3.0; Graph Pad Software Inc. San Diego, CA, USA) with the significance of the difference between the groups assessed using a one-way ANOVA, followed by Tukey *post hoc* means comparison test or a 2-way ANOVA. IC_{50}s were determined by regression analysis using Origin Software (OriginLab, Northampton, MA). Molecular docking of active polyphenolics to the known structure of human HDAC2 was conducted using lead optimization software - Mcule, Inc. (Palo Alto, CA, USA).

Results

Method validation and study setup. A series of method validation studies were first performed on human HDAC nuclear lysates to determine activity by product formation over time, signal of blanks and inhibition by a known HDAC inhibitor (Figure 1). Approximately 2-3 h of incubation at 37˚C was ample to drive de-acetylated peptide product with sufficient signal/noise ratio for fluorometric detection, being inhibited by a known HDACi (Figure 2), effects which occurred over a dose response presented alongside both negative and positive controls (Figure 3). Next a full panel

Table I. *Biological consequence of HeLa nuclear HDAC enzyme inhibition on the 24 h cell viability [0.5×10^5 cells/ml] and 3-4 day cell proliferation [T^0=0.04×10^5 cells/ml] of Hela cells. The data represent HDAC HeLa nuclear enzyme activity (IC_{50}), cell cytotoxicity (LC_{50}) and cytostatic (IC_{50}) effects in µg/mL, determined from linear regression of 6 concentrations , n=4.*

Biological effects – HeLa cells	IC_{50} HDAC	LC_{50} Viability	IC_{50} Proliferation	Units µg/mL
Paclitaxel [No Effect]	>25^{UL}	>25^{UL}	0.005	µg/mL
Trichostatin A	0.028	>25^{UL}	0.25	µg/mL
Apicidin	0.100	>25^{UL}	0.55	µg/mL
Superohydoxamic acid	0.282	>25^{UL}	8.92	µg/mL
M344	0.436	>25^{UL}	1.38	µg/mL
CL-994	0.520	>25^{UL}	2.60	µg/mL
Valproic Acid	2.160	>25^{UL}	>25^{UL}	µg/mL
Sodium phenylbutyrate	4.710	>25^{UL}	>25UL	µg/mL

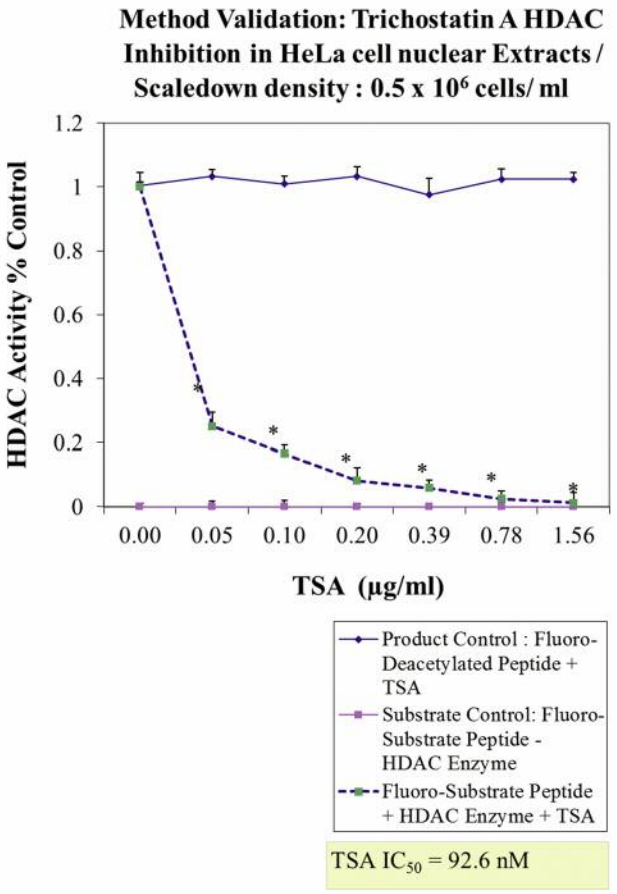

Figure 3. *HDAC enzyme activity assay method validation. A 3-h end-point was sufficient to evaluate HDAC enzyme activity in crude HeLa cell nuclear lysates, which was inhibited by Trichostatin A (TSA) (HDAC inhibitor), with no decay in solvent product control (-Enzyme) and no rise in RFU with substrate (-Enzyme). The data represent HDAC activity (% control) and are presented as the mean±S.E.M, n=4. Differences between control and TSA-treated samples were analyzed by a one-way ANOVA, followed by a Tukey post-hoc test. *p<0.05.*

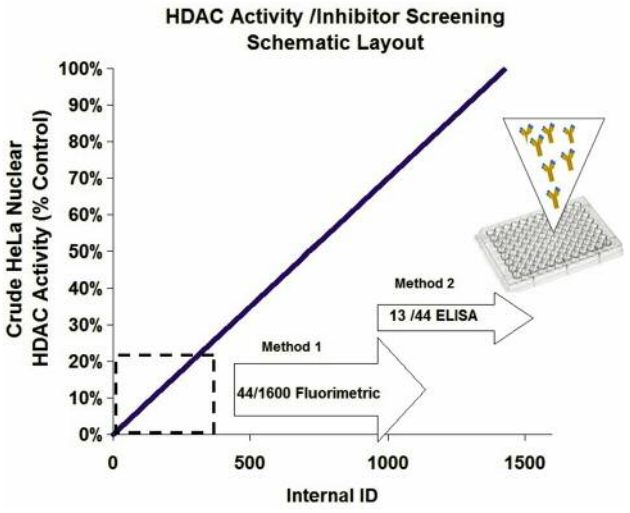

Figure 4. *High-throughput schematic. ~1,600 natural plant extracts (spices, herbs, teas, vegetables, fruits, seeds, rinds etc. (<600 µg/mL) and polyphenolics (<240 µg/ml) were screened for HDAC activity inhibition in nuclear HeLa cell lysates. Compounds demonstrating an IC_{20} at Tier 1 concentrations using Method 1 (fluorometric) were re-evaluated over a minimum of 6 concentrations and IC_{50}s were established. Dual validation was necessary as many natural compounds lead to significant interference with the developer solution in Method 1. Method 2 analysis eliminated the majority as false positives, with only a few natural products confirmed as HDAC inhibitors.*

Table II. *Summary of Changes: Grapeseed Extract and TSA (HDAC) inhibitor vs. controls using Affymetrix Expression Console Software and Affymetrix® Transcriptome Analysis Console (TAC) Software.*

Array Type: miRNA-4_1: Transcriptome of HeLa Cells		
Total number of genes	6631	
Differential Expressed: Contol *vs.* GSE	81	up-regulated
Differential Expressed: Contol *vs.* GSE	0	down-regulated
Differential Expressed: Contol *vs.* TSA	35	up-regulated
Differential Expressed: Contol *vs.* TSA	0	down-regulated

of HDAC inhibitors were evaluated under uniform conditions and IC_{50}s were established (Table I). Table I also presents biological effects of panel HDAC drugs comparing relative HDACi inhibition in nuclear lysates (IC_{50}s) with respective LC_{50} (toxicity) and IC_{50} (cytostatic-growth inhibition) effects in HeLa Cells. These findings show a predominant anti-mitotic effect rather than cytotoxic effect by known HDAC inhibitors.

Once validated, the HTP screening was conducted on over 1,600 natural products including herbs, seeds, plants, roots, fruits, vegetables, vitamins, organic metabolites, minerals, and drugs for potential HDAC inhibition in nuclear lysates (Figure 4). A 1st tier screening was used to evaluate all compounds at one concentration, to which any compound demonstrating an IC_{20} less than 600 µg/ml (for crude herbs) and 240 µg/ ml (for all individuals compounds) were re-evaluated in a 2nd screening. Second tier screenings on positive hits were acquired for 10 concentrations (dose response) to establish IC_{50}s, with solvent controls run for each compound at varying concentration. Tier 1 and 2 screenings used a common fluorometric activity assay [ab156064] (Abcam Cambridge, MA); Method 1. A major methodological concern was the numerous false positives arising due to inhibition of natural products on the lysine developer reagent (which itself contains an enzyme). Because of numerous interferences associated with Method

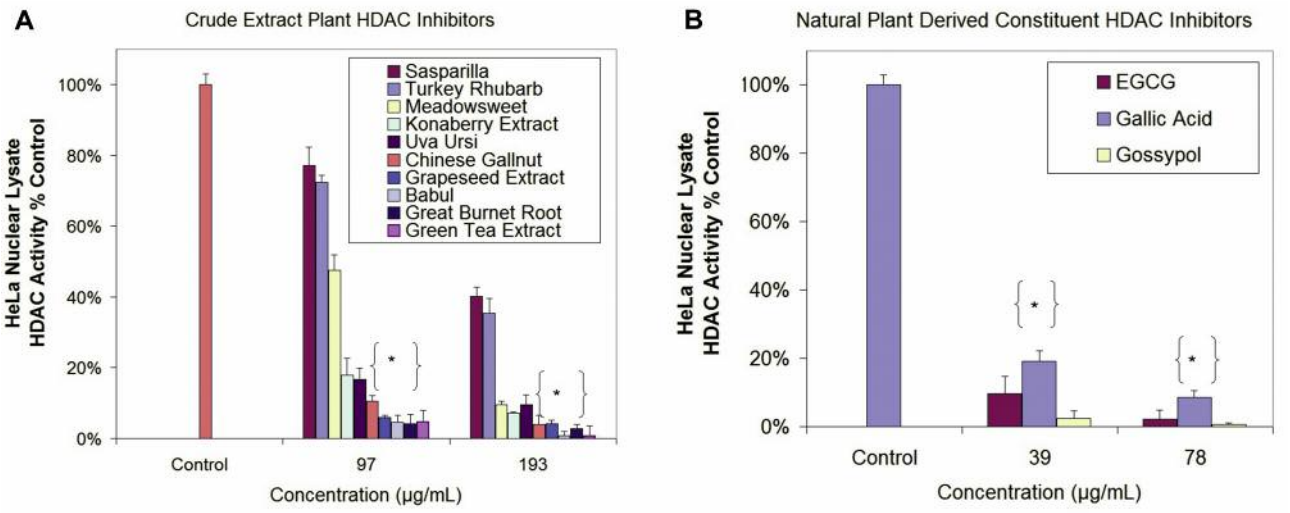

Figure 5. *(A,B). Plant based (A) and polyphenolic (B) HDAC inhibitors in HeLa nuclear lysates as determined using the direct specific-activity linked ELISA method. The data represented HDAC Activity as % controls and presented as the mean±S.E.M, n=3. Differences between control and treated samples were analyzed by a one-way ANOVA, followed by a Tukey post hoc test. *p<0.05.*

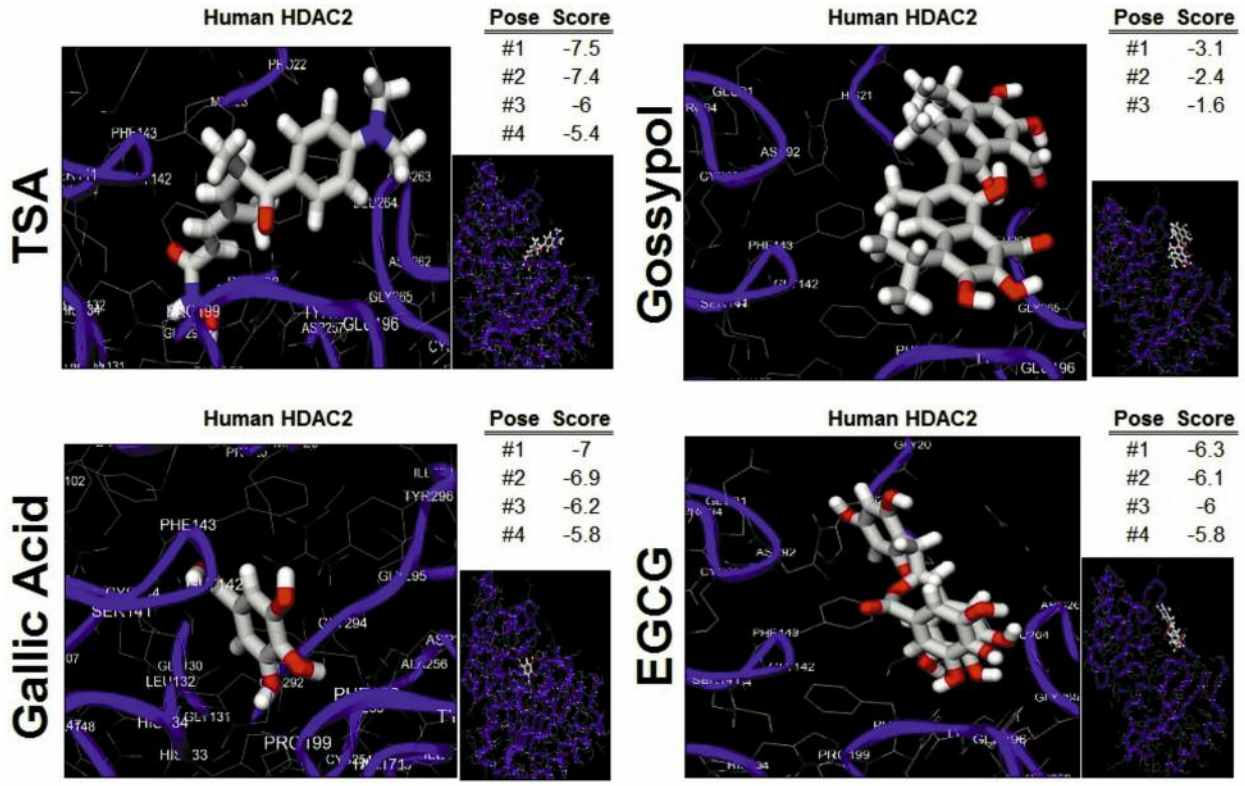

Figure 6. *Molecular docking of TSA vs. EGCG, gallic acid and gossypol to human HDAC 2.*

1, potential hits were re-validated using Method 2, an ELISA based HDAC activity/ inhibition direct assay #P-4035. Epigentek Group Inc. (Farmingdale, NY, USA).The data show very few natural products (13) as true HDACis at therapeutic doses (Figure 5A and B). Molecular docking of HDACi polyphenolics: gossypol, EGCG and gallic acid *vs*. TSA, a potent and irreversible inhibitor of human HDAC are presented in Figure 6.

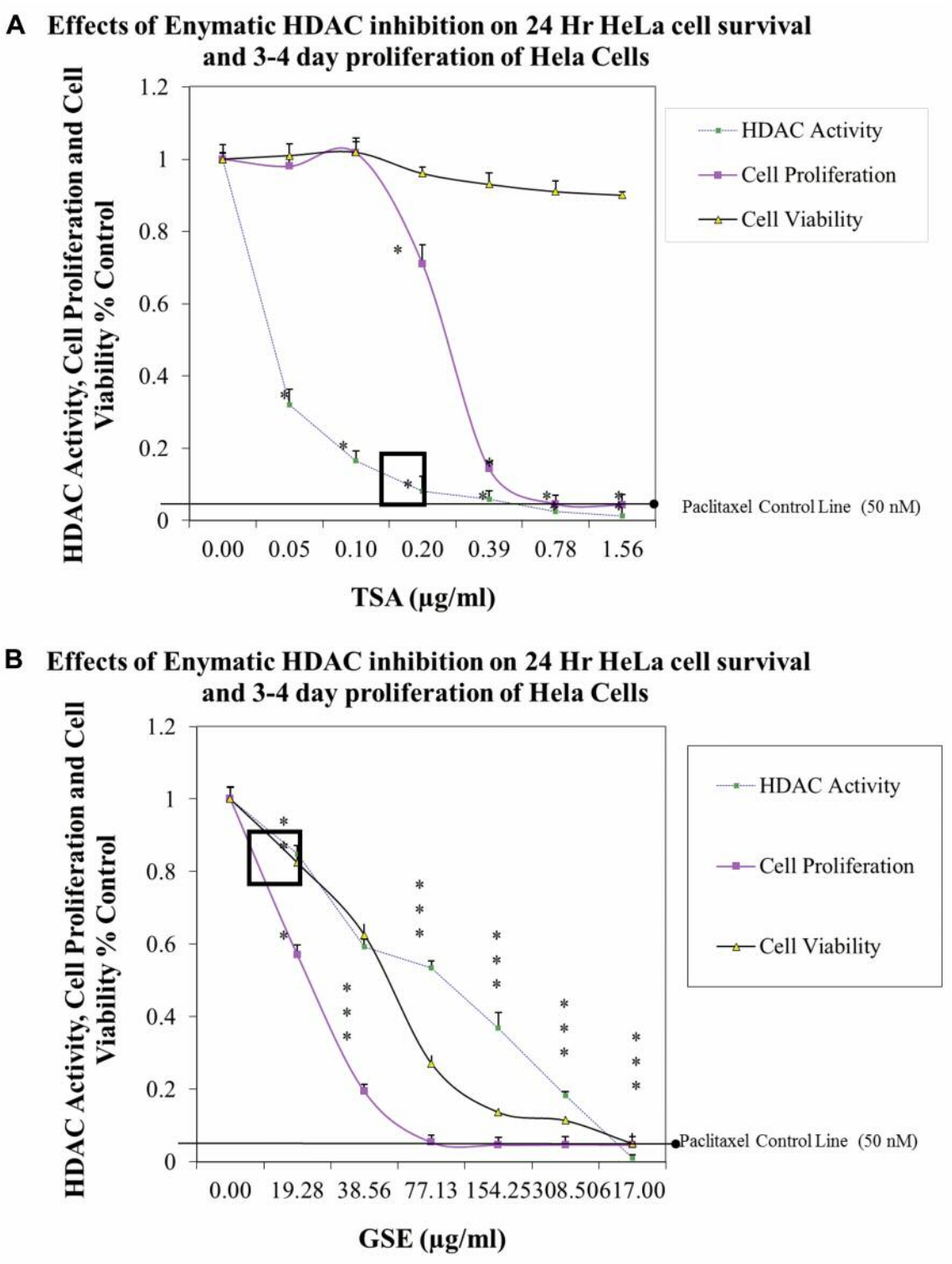

Figure 7. *A. Correlation between TSA inhibition of Hela cell HDAC enzyme activity and 24 cell viability [0.5×10^5 cells/ml] and 3-4 day cell proliferation [0.04×10^5 cells/ml] vs. paclitaxel (50 nM) in HeLa cells. The data represent HDAC enzyme activity, cell viability and proliferation as % controls, and are presented as the mean±S.E.M, n=4. Differences between control and TSA-treated samples were analyzed by a one-way ANOVA, followed by a Tukey post hoc test. *p<.05. B. Evaluation of GSE on 24-h cell viability [0.5×10^5 cells/ml] and 3-4 day cell proliferation [T0=0.04×10^5 cells/ml]. The data represent HDAC enzyme activity, cell viability and proliferation as % controls, and are presented as the mean±S.E.M, n=4. Differences between control and GSE treated samples were analyzed by a one-way ANOVA, followed by a Tukey post hoc test. *p<0.05.*

Table III. *List of elevated miRNA in TSA-treated Hela cells vs. controls: The data include fold change, Transcript ID, Accession Number, and p-Value.*

	Up-regulated miRNA by TSA			Bi-weight Avg Signal (log2)			
	Transcript Cluster ID	Accession	Transcript ID	Control	TSA	Fold Change	*p*-Value
1	20517821	MIMAT0017991	hsa-miR-3613-3p	5.28	0.98	19.79	0.01
2	20519463	MIMAT0019745	hsa-miR-4668-5p	4.92	1.96	7.77	0.01
3	20500720	MIMAT0004587	hsa-miR-23b-5p	4.33	1.38	7.71	0.01
4	20500722	MIMAT0004588	hsa-miR-27b-5p	3.25	0.67	5.98	0.04
5	20506712	MIMAT0005825	hsa-miR-1180-3p	2.79	0.88	3.77	0.00
6	20525721	MIMAT0027660	hsa-miR-6880-5p	3.82	1.99	3.55	0.05
7	20525746	MIMAT0027685	hsa-miR-6892-3p	2.66	0.87	3.46	0.03
8	20519672	MIMAT0019949	hsa-miR-4785	2.53	0.94	3.01	0.04
9	20500120	MIMAT0004484	hsa-let-7d-3p	1.9	0.32	2.98	0.03
10	20500795	MIMAT0004614	hsa-miR-193a-5p	5.6	4.16	2.72	0.03
11	20538197	U50B	U50B	1.58	0.22	2.57	0.03
12	20504340	MIMAT0003268	hsa-miR-600	1.22	–0.13	2.54	0.02
13	20535697	MI0005768	hsa-mir-943	1.12	–0.19	2.48	0.05
14	20500139	MIMAT0000075	hsa-miR-20a-5p	7.03	5.72	2.47	0.05
15	20519642	MIMAT0019919	hsa-miR-4767	1.53	0.26	2.42	0.05
16	20525430	MIMAT0027369	hsa-miR-6734-5p	1.31	0.06	2.39	0.03
17	20535843	MI0006367	hsa-mir-1302-6	1.15	–0.09	2.36	0.05
18	20536916	MI0017318	hsa-mir-4686	1.91	0.69	2.32	0.04
19	20538310	ENSG00000252299	ENSG00000252299	2	0.8	2.3	0.04
20	20534343	MI0000063	hsa-let-7b	1.31	0.11	2.3	0.04
21	20506002	MIMAT0004976	hsa-miR-933	1.98	0.79	2.28	0.04
22	20518793	MIMAT0018936	hsa-miR-4423-3p	1.07	–0.07	2.21	0.03
23	20518865	MIMAT0019006	hsa-miR-4478	1.24	0.14	2.14	0.03
24	20500130	MIMAT0000070	hsa-miR-17-5p	7.77	6.69	2.12	0.02
25	20525457	MIMAT0027396	hsa-miR-6748-5p	3	1.93	2.11	0.02
26	20500115	MIMAT0000063	hsa-let-7b-5p	10.74	9.67	2.1	0.05
27	20500440	MIMAT0000254	hsa-miR-10b-5p	0.9	–0.16	2.08	0.04
28	20536854	MI0017261	hsa-mir-4634	1.6	0.55	2.07	0.03
29	20525631	MIMAT0027570	hsa-miR-6835-5p	1.27	0.23	2.06	0.02
30	20533875	ENSG00000252112	ENSG00000252112	1.12	0.09	2.04	0.03
31	20537471	MI0022572	hsa-mir-6727	1.37	0.34	2.03	0.03
32	20536813	MI0016900	hsa-mir-4533	1.22	0.2	2.03	0.04
33	20533208	ENSG00000238316	ENSG00000238316	0.78	–0.23	2.02	0.01
34	20518937	MIMAT0019075	hsa-miR-4535	0.99	–0.03	2.02	0.03
35	20525446	MIMAT0027385	hsa-miR-6742-5p	0.9	–0.11	2.01	0.04

In order to determine if there are any similar influences between an identified plant HDACi and a known HDACi (TSA), we chose grape seed extract (GSE) for further studies. First, the biological effects of GSE and TSA on Hela cells with respective HDAC inhibition (nuclear lysates) are presented in Figure 7A and B. Dual validation of HDAC inhibition by both methods are presented in Figure 8. To ensure GSE as a true HDACi; we tested numerous supplier batches of GSE from diverse vendors including Bulk Supplements.com/Hard Eight Nutrition LLC (Henderson, NV, USA), Futureceuticals (Momence, IL, USA), and capsules obtained at General Nutrition Center (GNC) and Swanson's (Fargo, ND, USA), all near identical effects.

To evaluate the epigenetic influence of HDACs on whole transcriptome miRNAs, we compared TSA *vs*. GSE (Bulk supplements (Henderson, NV, USA) at sub-lethal doses (20 μg/ml GSE and 0.2 μg/ml TSA) with samples collected at 24 h –for whole genomic evaluation of small non-coding RNA using Affymetrix' miRNA 4.1 Arrays/FlashTag™ Labeling. The data show that at baseline, there were 6,631 miRNAs expressed in HELA cells, to which 35 were up-regulated by TSA and 81 genes up-regulated by GSE (Table II). Changes by TSA on specific miRNAs *vs*. controls in HeLa cells are provided in Table III (fold change) and in Table IV (showing the KEGG pathway association and miRNA target genes). Likewise, changes by GSE on specific miRNAs vs controls in HeLa cells are provided in Table V (fold change) and in Table VI (showing information on KEGG pathway association and miRNA target genes). Corresponding biological target heat maps showing an

Table IV. *Full list of elevated miRNA changes in TSA-treated Hela cells vs controls: The data include miRNAs, KEGG Pathway association, target genes and p-Value.*

TSA HDAC inhibitor mediated miRNA up-regulation *vs*. control

	KEGG pathway	*p*-Value	miRNAS	Target Genes
1	Pathways in cancer (hsa05200)	<1e-16	hsa-miR-20a-5p|Tarbase hsa-miR-17-5p|Tarbase	E2F1, NRAS, RUNX1, BCL2, MAPK9, CCND1, SMAD4, HIF1A, MYC, CDKN1A, VEGFA, PTEN, TGFBR2, JAK1
2	Bladder cancer (hsa05219)	1.12E-10	hsa-miR-20a-5p|Tarbase hsa-miR-17-5p|Tarbase hsa-let-7b-5p|Tarbase	E2F1, NRAS, MAP2K2, THBS1, KRAS, CCND1, MYC, CDKN1A, VEGFA
3	Chronic myeloid leukemia (hsa05220)	1.4E-08	hsa-miR-20a-5p|Tarbase hsa-miR-17-5p|Tarbase hsa-let-7b-5p|Tarbase	E2F1, NRAS, CRKL, MAP2K2, RUNX1, KRAS, CDK6, CCND1, MYC, SMAD4, CDKN1A, TGFBR2
4	Hepatitis B (hsa05161)	2.54E-07	hsa-miR-20a-5p|Tarbase hsa-miR-17-5p|Tarbase	E2F1, NRAS, BCL2, MAPK9, CCND1, SMAD4, MYC, CDKN1A, PTEN. JAK1
5	Colorectal cancer (hsa05210)	2.42E-06	hsa-miR-20a-5p|Tarbase hsa-miR-17-5p|Tarbase	BCL2, MAPK9, CCND1, SMAD4, MYC, TGFBR2
6	Melanoma (hsa05218)	0.000881	hsa-miR-20a-5p|Tarbase hsa-miR-17-5p|Tarbase hsa-let-7b-5p|Tarbase	E2F1, NRAS, MAP2K2, KRAS, CDK6, CCND1, CDKN1A, PTEN
7	Pancreatic cancer (hsa05212)	0.003214	hsa-miR-20a-5p|Tarbase hsa-miR-17-5p|Tarbase	E2F1, MAPK9, CCND1, SMAD4, VEGFA, E2F1, TGFBR2, JAK1
8	Glioma (hsa05214)	0.003683	hsa-miR-20a-5p|Tarbase hsa-miR-17-5p|Tarbase hsa-let-7b-5p|Tarbase	PDGFRA, E2F1, NRAS, MAP2K2, KRAS, CDK6, CCND1, CDKN1A, PTEN
9	TGF-beta signaling pathway (hsa04350)	0.003735	hsa-miR-20a-5p|Tarbase hsa-miR-17-5p|Tarbase	THBS1, SMAD4, MYC, TGFBR2, BMPR2
10	Prostate cancer (hsa05215)	0.010164	hsa-miR-20a-5p|Tarbase hsa-miR-17-5p|Tarbase hsa-let-7b-5p|Tarbase	PDGFRA, E2F1, NRAS, MAP2K2, BCL2, KRAS, CCND1, PDK1, CDKN1A, PTEN

integration of biological processes associated with changes in miRNAs are shown in Figure 9 for TSA and Figure 10 for GSE. Similar patterned miRNA changes by both GSE and TSA-treated HeLa cells are provided in Table VII. The KEGG pathway for cancer signaling is presented in Figure 11 to which targeted genes for all miRNAs are highlighted in red (TSA) and purple (GSE). These findings demonstrate a rather consistent effect on cancer signaling between a known HDAC drug (TSA) and GSE.

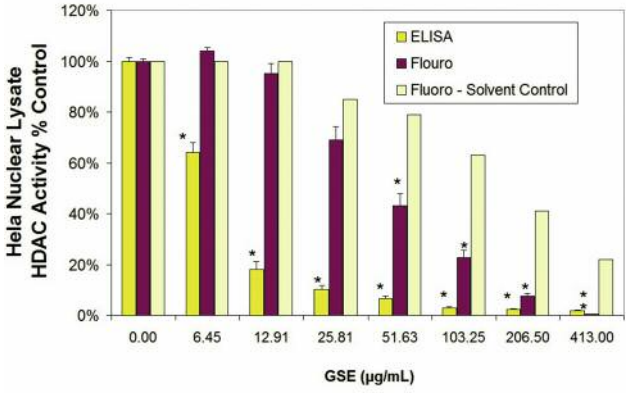

Figure 8. *Effects of GSE on HDAC activity comparing a Method 1 (fluorometric) vs. Method 2 (sensitive and specific - ELISA based HDAC Activity/Inhibition Assay). The data represents HDAC Activity as % controls and are presented as the mean±S.E.M, n=3. Differences between Control and GSE treated samples were analyzed by a one-way ANOVA, followed by a Tukey post hoc test. *p<0.05.*

Discussion

HDAC inhibitors are becoming a well-known class of drugs for cancer treatment, in particular after the FDA approval of Vorinostat and Romidepsin for the treatment of hematologic cancers (2). At least 12 different HDACis are currently in use for clinical trials, and more are being sought to augment chemotherapy (4, 5, 7), immunotherapy/ cancer vaccines (14) and overcome multi-drug resistance which are common to diverse cytostatic or receptor-mediated drugs (3, 8, 9, 36-38). Further, HDACis can prevent metastasis (24) attenuate epithelial-mesenchymal transition (11, 12) influence the re-expression of tumor suppressors (22) attenuate oncogenes (26) and sustain greater negative impact on targets such as PI3K/AKT/mTOR pathway (28).

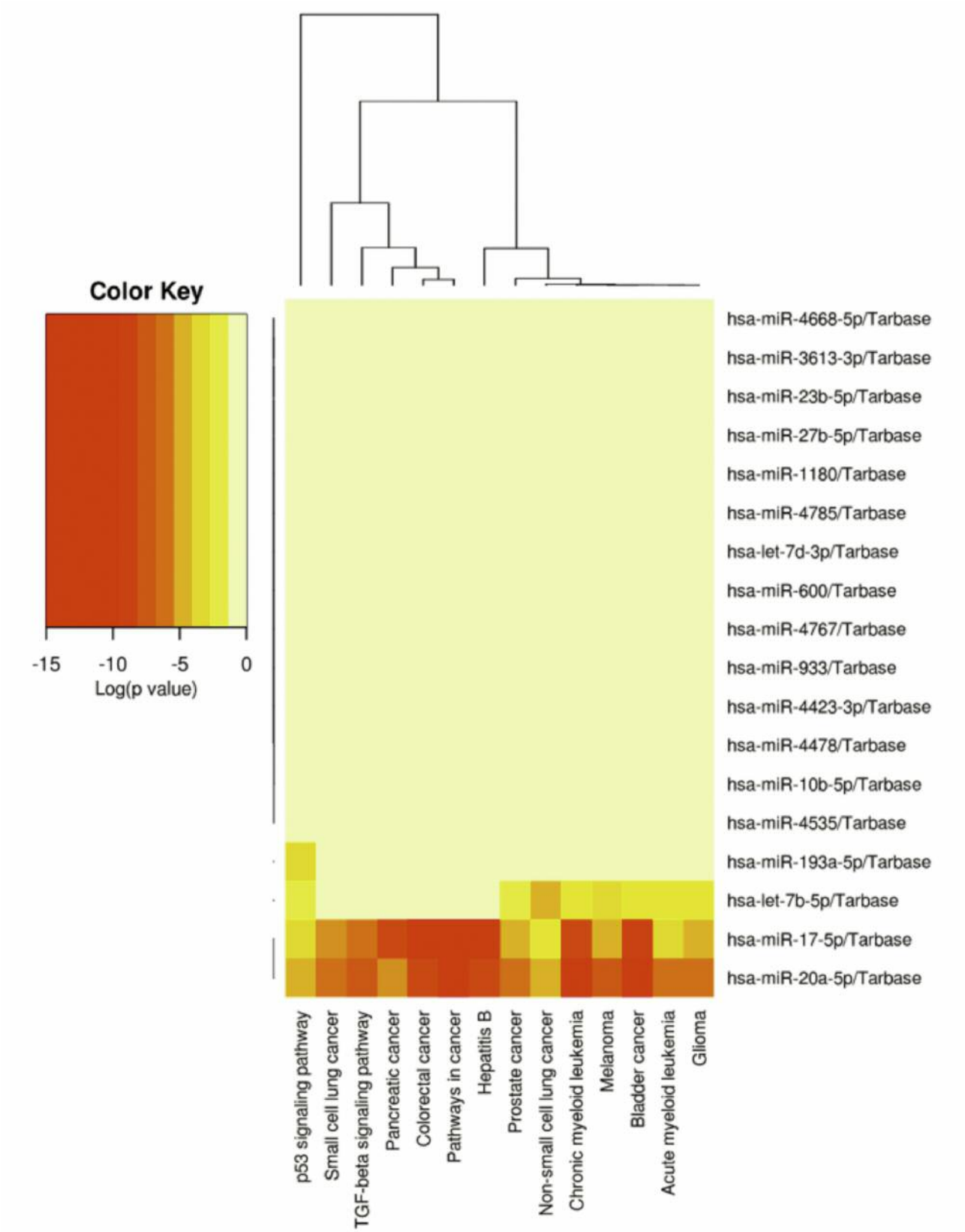

Figure 9. *Biological Target (Heatmap) and corresponding Table IV miRNA profile from Pathway Integration of miRNA target genes using DIANA TOOLS - mirPath v.3. TSA vs. Controls in HeLa cells.*

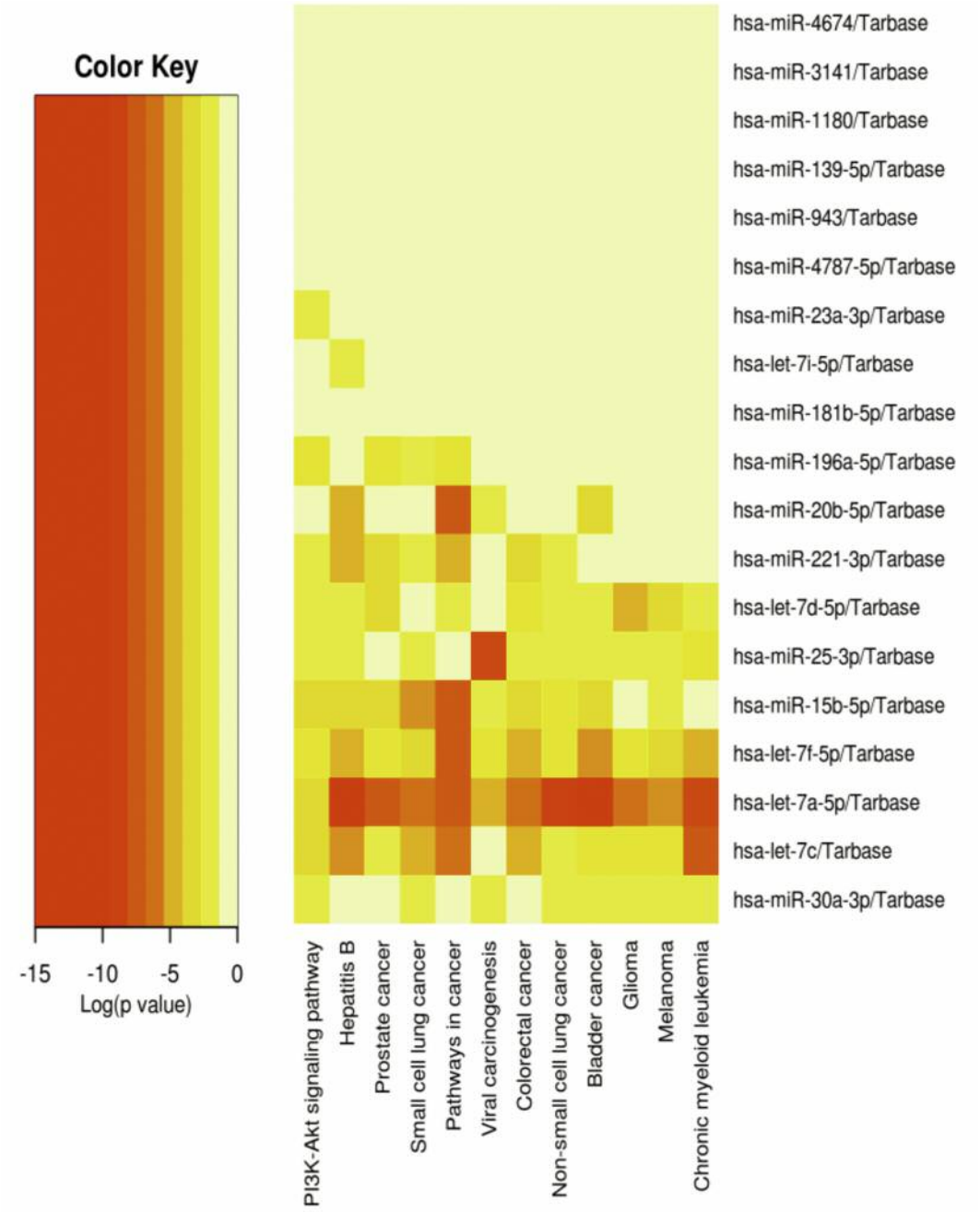

Figure 10. *Biological (Heat map) and corresponding Table VI miRNA profile from Pathway Integration of miRNA target genes using DIANA TOOLS - mirPath v.3. GSE vs. Controls in HeLa cells.*

HDAC inhibitors were first isolated from microorganisms, and continue to be developed from microbial metabolites (39, 40). Likewise, endogenous microbial fermentation of non-digestible starches in gut microflora can release HDACi butyrate – believed to prevent colon cancer (31, 32). Therefore there remains doubt as to if plant based HDACis exist and if so, how they compare with microbial based HDACis.

In this study, we conducted a HTP screening under uniform experimental conditions to identify plant-based or commonly used nutraceuticals with HDAC inhibitory properties. It is imperative to note, unlike many other screenings we have previously conducted, that we faced considerable challenges using common commercially available methodologies to evaluate enzymatic HDAC activity. A major challenge was the

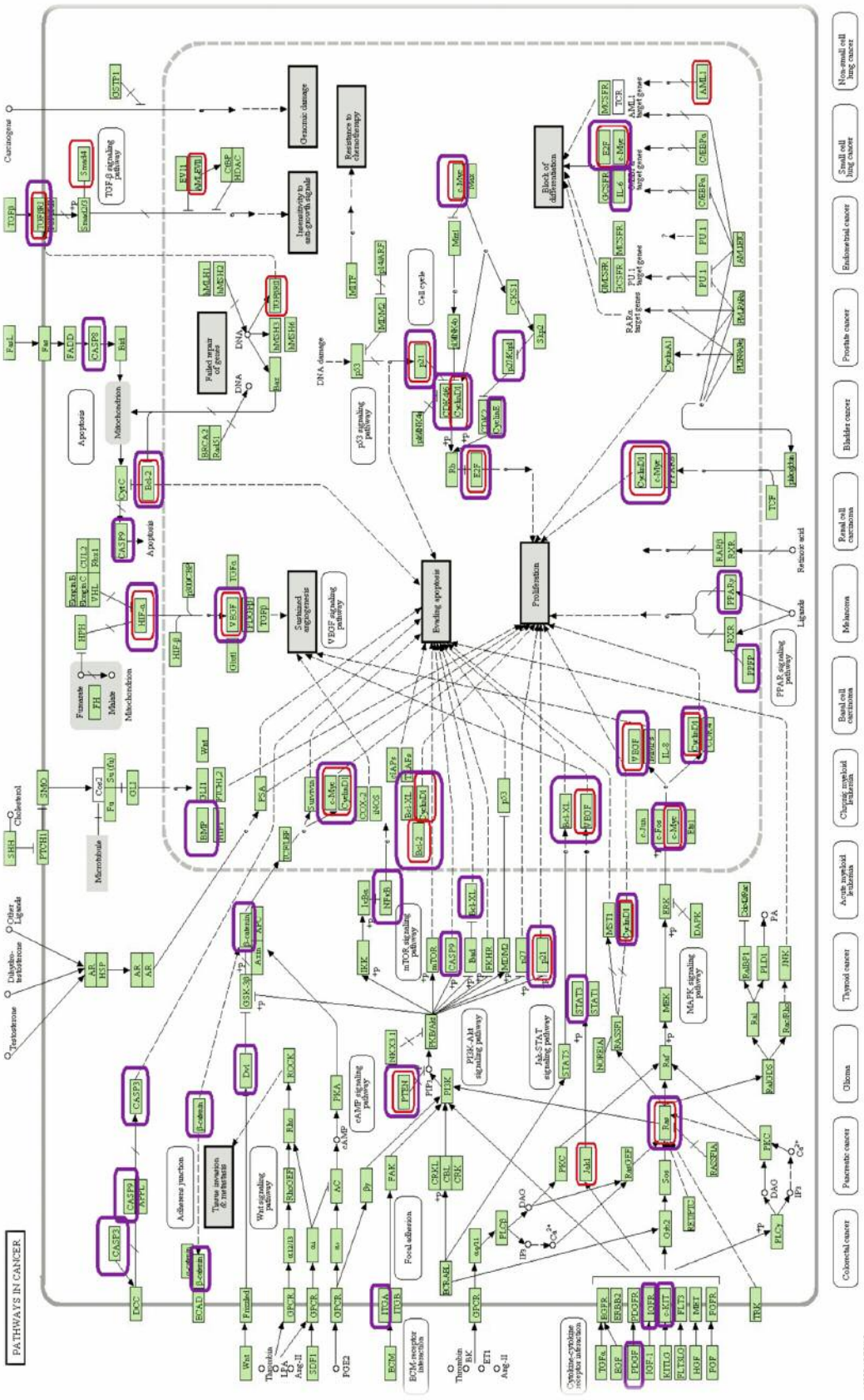

Figure 11. *Similar miRNA up-regulation by GSE and TSA in HeLa cells with predicted target genes overlapped on a KEGG "Pathways in Human Cancer" schematic. Red=TSA, purple=GSE where red+purple=TSA and GSE combined targets.*

Table V. *List of elevated miRNA in GSE-treated Hela cells vs. controls: The data include fold change, Transcript ID, Accession Number, and p-Value.*

	Up-regulated miRNA by GSE			Bi-weight Avg Signal (log2)			
	Transcript Cluster ID	Accession	Transcript ID	Control	GSE	Fold Change	*p*-value
1	20525453	hsa-miR-6746-5p	MIMAT0027392	2.86	-0.12	7.87	0.00
2	20519576	hsa-miR-4732-5p	MIMAT0019855	3.25	0.28	7.78	0.05
3	20500720	hsa-miR-23b-5p	MIMAT0004587	4.33	1.59	6.7	0.05
4	20506872	hsa-miR-1275	MIMAT0005929	4.34	1.61	6.63	0.03
5	20518935	hsa-miR-4534	MIMAT0019073	4.14	1.44	6.51	0.05
6	20525721	hsa-miR-6880-5p	MIMAT0027660	3.82	1.14	6.43	0.02
7	20500722	hsa-miR-27b-5p	MIMAT0004588	3.25	0.59	6.32	0.04
8	20504379	hsa-miR-629-5p	MIMAT0004810	3.58	1.03	5.86	0.04
9	20524053	hsa-miR-6132	MIMAT0024616	3.88	1.47	5.31	0.04
10	20518432	hsa-miR-3911	MIMAT0018185	4.99	2.68	4.94	0.05
11	20500393	hsa-miR-196a-5p	MIMAT0000226	4.48	2.21	4.84	0.04
12	20518807	hsa-miR-4433-3p	MIMAT0018949	4.3	2.05	4.76	0.03
13	20525503	hsa-miR-6771-5p	MIMAT0027442	3.9	1.73	4.52	0.03
14	20510799	hsa-miR-1972	MIMAT0009447	2.93	0.8	4.37	0.05
15	20538194	U49A	U49A	5.08	2.96	4.33	0.05
16	20500446	hsa-miR-181b-5p	MIMAT0000257	5.24	3.19	4.16	0.01
17	20515627	hsa-miR-320e	MIMAT0015072	4.74	2.78	3.87	0.04
18	20519417	hsa-miR-4640-5p	MIMAT0019699	1.95	0.01	3.83	0.04
19	20500163	hsa-miR-30a-3p	MIMAT0000088	3.94	2.01	3.83	0.05
20	20500394	hsa-miR-197-5p	MIMAT0022691	3.76	1.83	3.82	0.02
21	20535295	hsa-mir-92b	MI0003560	3.83	1.92	3.76	0.02
22	20519441	hsa-miR-4656	MIMAT0019723	4.28	2.37	3.76	0.04
23	20502237	hsa-miR-20b-5p	MIMAT0001413	4.38	2.53	3.62	0.01
24	20538140	U17b	U17b	6.36	4.55	3.51	0.03
25	20517736	hsa-miR-4281	MIMAT0016907	5.45	3.67	3.45	0.00
26	20500112	hsa-let-7a-5p	MIMAT0000062	9.15	7.38	3.4	0.01
27	20500120	hsa-let-7d-3p	MIMAT0004484	1.9	0.14	3.38	0.00
28	20500484	hsa-miR-221-3p	MIMAT0000278	6.12	4.37	3.38	0.01
29	20524034	hsa-miR-6124	MIMAT0024597	2.75	1.02	3.32	0.02
30	20525565	hsa-miR-6802-5p	MIMAT0027504	3.36	1.66	3.24	0.01
31	20536582	hsa-mir-3679	MI0016080	2.35	0.68	3.19	0.03
32	20537087	hsa-mir-5095	MI0018001	2.81	1.15	3.17	0.05
33	20519433	hsa-miR-4651	MIMAT0019715	5.17	3.55	3.07	0.00
34	20500117	hsa-let-7c-5p	MIMAT0000064	8.93	7.32	3.06	0.02
35	20519626	hsa-miR-4758-5p	MIMAT0019903	3.87	2.27	3.03	0.03
36	20534220	HBII-135	HBII-135	3.17	1.6	2.99	0.01
37	20500151	hsa-miR-25-3p	MIMAT0000081	4.38	2.82	2.95	0.02
38	20506006	hsa-miR-937-5p	MIMAT0022938	4.34	2.8	2.91	0.02
39	20533073	ENSG00000212378	ENSG00000212378	6.51	4.97	2.9	0.00
40	20538252	U78	U78	6.51	4.97	2.9	0.00
41	20519488	hsa-miR-4684-3p	MIMAT0019770	1.7	0.17	2.87	0.01
42	20500123	hsa-let-7f-5p	MIMAT0000067	6.2	4.69	2.85	0.03
43	20538192	U49A	U49A	3.56	2.1	2.76	0.05
44	20538195	U49B	U49B	3.56	2.1	2.76	0.05
45	20538247	U75	U75	3.93	2.48	2.73	0.01
46	20526178	hsa-miR-7110-5p	MIMAT0028117	3.77	2.33	2.7	0.02
47	20525587	hsa-miR-6813-5p	MIMAT0027526	4.13	2.69	2.7	0.02
48	20504341	hsa-miR-601	MIMAT0003269	1.86	0.44	2.68	0.01
49	20519636	hsa-miR-4763-3p	MIMAT0019913	5.63	4.25	2.61	0.04
50	20538244	U73a	U73a	4.67	3.3	2.59	0.03
51	20501286	hsa-miR-151a-5p	MIMAT0004697	6.55	5.18	2.59	0.04
52	20538253	U78	U78	6.19	4.83	2.57	0.01
53	20538193	U49A	U49A	4.84	3.5	2.53	0.05
54	20519474	hsa-miR-4674	MIMAT0019756	4.67	3.35	2.5	0.04
55	20538254	U79	U79	3.48	2.16	2.49	0.05
56	20538248	U75	U75	3.89	2.59	2.46	0.02

Table V. *Continued*

Table V. *Continued*

	Up-regulated miRNA by GSE			Bi-weight Avg Signal (log2)			
	Transcript Cluster ID	Accession	Transcript ID	Control	GSE	Fold Change	*p*-value
57	20515550	hsa-miR-3141	MIMAT0015010	6.49	5.2	2.46	0.03
58	20525559	hsa-miR-6799-5p	MIMAT0027498	1.7	0.42	2.43	0.00
59	20538199	U50	U50	5.06	3.78	2.42	0.01
60	20538159	U30	U30	5.17	3.9	2.41	0.01
61	20500146	hsa-miR-23a-3p	MIMAT0000078	9.66	8.39	2.4	0.03
62	20506712	hsa-miR-1180-3p	MIMAT0005825	2.79	1.55	2.37	0.04
63	20525491	hsa-miR-6765-5p	MIMAT0027430	5.8	4.56	2.36	0.02
64	20538300	mgh28S-2411	mgh28S-2411	4.8	3.56	2.36	0.03
65	20538249	U76	U76	7.8	6.58	2.33	0.04
66	20523000	hsa-miR-6068	MIMAT0023693	2.72	1.53	2.28	0.01
67	20500432	hsa-miR-139-5p	MIMAT0000250	0.99	-0.18	2.25	0.03
68	20535697	hsa-mir-943	MI0005768	1.12	-0.04	2.24	0.04
69	20500718	hsa-miR-15b-5p	MIMAT0000417	6.06	4.9	2.24	0.04
70	20537261	hsa-mir-6089-1	MI0020366	3.85	2.71	2.2	0.02
71	20537862	hsa-mir-6089-2	MI0023563	3.85	2.71	2.2	0.02
72	20519679	hsa-miR-4787-5p	MIMAT0019956	9.25	8.15	2.16	0.05
73	20525416	hsa-miR-6727-5p	MIMAT0027355	7.88	6.77	2.16	0.05
74	20538197	U50B	U50B	1.58	0.49	2.13	0.03
75	20500715	hsa-let-7i-5p	MIMAT0000415	6.67	5.58	2.12	0.00
76	20538255	U80	U80	1.88	0.82	2.09	0.04
77	20500119	hsa-let-7d-5p	MIMAT0000065	8.26	7.2	2.09	0.04
78	20523021	hsa-miR-6089	MIMAT0023714	8.93	7.89	2.06	0.04
79	20532675	ACA44	ACA44	5.34	4.32	2.03	0.01
80	20534056	ENSG00000252840	ENSG00000252840	5.34	4.32	2.03	0.01
81	20529779	hsa-miR-8069	MIMAT0030996	8.37	7.36	2.02	0.03

numerous false-positives which originated from the inhibition of natural products on fluorimetric lysine developer reagents (which themselves contain an enzyme). The inability to ascertain HDACi due to solvent decay required validation using a more robust and sensitive activity-based method. With dual detection methods, we were able to ascertain only 13 plant-based HDACi which included grapeseed extract (GSE), great burnet root (*Sanguisorba Officinalis*), Babul (*Acacia arabica*), Chinese gallnut (*Melaphis Chinensis*), konaberry extract (*Coffea arabica*), Uva Ursi (*Arctostaphylos uva ursi*), epigallocatechin gallate (EGCG), green tea (*Camellia sinensis*), meadowsweet (*Filipendula ulmaria*), sassafras (*Sassafras Officinale*), Turkey Rhubarb (*Rheum palmatum*), gossypol and gallic acid.

Grapeseed extract. GSE has an extensive history in chemoprevention, yet there are very few studies demonstrating its influence on the epigenome. *In vivo* studies have demonstrated the capacity of dietary intake of GSE proanthocyanidins (0.2% and 0.5%, w/w) to attenuate DNA damage by UV radiation-induced as well as attenuate advanced melanoma, malignant transformation of papilloma to carcinoma (41-43) and other diverse tumors (*e.g.*, hormone-refractory human prostate carcinoma) (44). These effects have also been observed in humans, elucidated by epidemiological studies such as the Vitamins And Lifestyle (VITAL) cohort study. In the VITAL study, dietary intake of GSE is inversely related to the risk of hematologic malignancies, possibly indicating a connection to its capacity to inhibit HDAC (45). Intake of GSE is also a correlate to reduced cutaneous squamous cell carcinoma and prostate cancer, while many other dietary supplements fail to show a correlation (46). While few reports exist on GSE and epigenetic targets, of those that do, they show influences on tumor suppressor microRNAs (47) global DNA methylation, DNA methyltransferase (DNMT) activity and expression, and changes in altered acetylated lysines H3 marks (48) in a similar demonstration to the results of this study.

The findings of the current study not only confirm major changes in microRNAs initiated by GSE similar to the known HDACi TSA, but also show that TSA and GSE lead to 6 overlapping cancer patterns on miRNAs with remarkable similarity in fold change - GSE/TSA fold up-regulation (hsa-let-7d-3p, 3.38/2.98, hsa-miR-23b-5p: 6.7/

Table VI. *Full list of elevated miRNA changes in GSE-treated Hela cells vs controls: The data include miRNAs, KEGG Pathway association, target genes and p-Value.*

GSE HDAC inhibitor mediated miRNA up-regulation *vs*. control				
	KEGG pathway	*p*-Value	miRNAS	Target Genes
1	Pathways in cancer (hsa05200)	6.59E-14	hsa-miR-196a-5p|Tarbase hsa-miR-20b-5p|Tarbase hsa-let-7a-5p|Tarbase hsa-miR-221-3p|Tarbase hsa-let-7c|Tarbase hsa-let-7f-5p|Tarbase hsa-miR-15b-5p|Tarbase hsa-let-7d-5p|Tarbase	FOS, STAT3, E2F1, TGFBR1, NFKB1, NRAS, BCL2, CDKN1B, IGF1R, KRAS, CDK6, ITGAV, CASP3, CCND1, CTNNB1, HIF1A, E2F3, MYC, KIT, CASP9, HRAS, PPARG, CASP8, BCL2L1, CCNE1, CDKN1A, VEGFA, PTEN, IL6, DVL2, BMP4, PDGFA,
2	Hepatitis B (hsa05161)	2.84E-13	hsa-miR-20b-5p|Tarbase hsa-let-7a-5p|Tarbase hsa-miR-221-3p|Tarbase hsa-let-7c|Tarbase hsa-miR-25-3p|Tarbase hsa-let-7f-5p|Tarbase hsa-miR-15b-5p|Tarbase hsa-let-7i-5p|Tarbase hsa-let-7d-5p|Tarbase	FOS, STAT3, E2F1, TGFBR1, NFKB1, E2F2, NRAS, BCL2, CDKN1B, TLR4, KRAS, CDK6, TICAM1, TP53, EGR3, CASP3, CCND1, E2F3, MYC, TBK1, CASP9, HRAS, CASP8, CCNE1, CDKN1A, PTEN, IL6
3	Bladder cancer (hsa05219)	3.24E-05	hsa-miR-30a-3p|Tarbase hsa-miR-20b-5p|Tarbase hsa-let-7a-5p|Tarbase hsa-let-7c|Tarbase hsa-miR-25-3p|Tarbase hsa-let-7f-5p|Tarbase hsa-miR-15b-5p|Tarbase hsa-let-7d-5p|Tarbase	E2F1, E2F2, NRAS, THBS1, KRAS, TP53, CCND1, MYC, HRAS, CDKN1A, VEGFA
4	Chronic myeloid leukemia (hsa05220)	9.82E-05	hsa-miR-30a-3p|Tarbase hsa-let-7a-5p|Tarbase hsa-let-7c|Tarbase hsa-miR-25-3p|Tarbase hsa-let-7f-5p|Tarbase hsa-let-7d-5p|Tarbase	E2F1, TGFBR1, NFKB1, E2F2, NRAS, KRAS, CDK6, TP53, CCND1, MYC, HRAS, BCL2L1, CDKN1A
5	Small cell lung cancer (hsa05222)	0.000839	hsa-miR-196a-5p|Tarbase hsa-miR-30a-3p|Tarbase hsa-let-7a-5p|Tarbase hsa-miR-221-3p|Tarbase hsa-let-7c|Tarbase hsa-miR-25-3p|Tarbase hsa-let-7f-5p|Tarbase hsa-miR-15b-5p|Tarbase	E2F1, NFKB1, E2F2 ,BCL2, CDKN1B, CDK6, TP53, ITGAV, CCND1, E2F3, MYC, CASP9, BCL2L1, CCNE1, PTEN
6	Prostate cancer (hsa05215)	0.00102	hsa-miR-196a-5p|Tarbase hsa-let-7a-5p|Tarbase hsa-miR-221-3p|Tarbase hsa-let-7c|Tarbase hsa-let-7f-5p|Tarbase hsa-miR-15b-5p|Tarbase hsa-let-7d-5p|Tarbase	E2F1, NFKB1, E2F2, NRAS, BCL2, CDKN1B, IGF1R, KRAS, CCND1, CTNNB1, E2F3, CASP9, HRAS, CCNE1, CDKN1A, PTEN, PDGFA
7	Colorectal cancer (hsa05210)	0.003297	hsa-let-7a-5p|Tarbase hsa-miR-221-3p|Tarbase hsa-let-7c|Tarbase hsa-miR-25-3p|Tarbase hsa-let-7f-5p|Tarbase hsa-miR-15b-5p|Tarbase hsa-let-7d-5p|Tarbase	FOS, TGFBR1, BCL2, KRAS, TP53, CASP3, CCND1, CTNNB1, MYC, CASP9
8	PI3K-Akt signaling pathway (hsa04151)	0.004919	hsa-miR-196a-5p|Tarbase hsa-miR-30a-3p|Tarbase	NFKB1, NRAS, THBS1, CCND2, BCL2, CDKN1B, IGF1R, KRAS, CDK6, TP53, ITGAV, DDIT4, CCND1,

Table VI. *Continued*

Table VI. *Continued*

GSE HDAC inhibitor mediated miRNA up-regulation *vs*. control				
	KEGG pathway	*p*-Value	miRNAS	Target Genes
9	Glioma (hsa05214)	0.020994	hsa-let-7a-5p|Tarbase hsa-miR-221-3p|Tarbase hsa-let-7c|Tarbase hsa-miR-25-3p|Tarbase hsa-let-7f-5p|Tarbase hsa-miR-23a-3p|Tarbase hsa-miR-15b-5p|Tarbase hsa-let-7d-5p|Tarbase hsa-miR-30a-3p|Tarbase hsa-let-7a-5p|Tarbase hsa-let-7c|Tarbase hsa-miR-25-3p|Tarbase hsa-let-7f-5p|Tarbase hsa-let-7d-5p|Tarbase	MYC, KIT, CASP9, HRAS, BCL2L1, CCNE1, FOXO3, CDKN1A, VEGFA, PTEN, IL6, BCL2L11, IL6R, PDGFA E2F1, E2F2, NRAS, IGF1R, KRAS, CDK6, TP53, CCND1, HRAS, CDKN1A, PDGFA
10	Viral carcinogenesis (hsa05203)	0.026785	hsa-miR-30a-3p|Tarbase hsa-miR-20b-5p|Tarbase hsa-let-7a-5p|Tarbase hsa-miR-25-3p|Tarbase hsa-let-7f-5p|Tarbase hsa-miR-15b-5p|Tarbase	STAT3, NFKB1, NRAS, CCND2, KRAS, CDK6, TP53, EGR3, CASP3, CCND1, HRAS, KAT2B, CASP8, CCNE1, CDKN1A

7.71, hsa-miR-27b-5p 6.32/5.98, hsa-miR-1180-3p 2.37/3.77, hsa-miR-6880-5p 6.43/3.55 and hsa-mir-943 2.24/2.48). For TSA, up-regulation of hsa-miR-27b-5p, hsa-miR-23b-5p, hsa-miR-3613-3p, hsa-miR-20a-5p and hsa-miR-17-5p are target-specific for destruction of oncogenes including E2F1, NRAS, BCL2, MAPK9, HIF1a, SMAD 4 , MYC, CDKN1a, VEGFA, JAK1, TGFBR2. In the case of GSE, greater than 4-fold elevation alone for miRNAs such as hsa-miR-23b-5p, hsa-miR-196a-5p, hsa-miR-629-5p, hsa-miR-1275 hsa-miR-27b-5p are predicted to down-regulate over 80 cancer pathway genes, including the aforementioned as well as STAT3, FOS, TGFBR1, NFKB1, E2F2,3, CDKN1B, BRAF, IGF1R, JUN, BIRC3 KRAS, CDK4 and 6, HRAS or invasive oncogenes such as ITGAV.

In summary, the data from this study show several natural product HDACis, and a consequential pattern associated with elevated tumor-suppressor miRNAs. While there is a wealth of information in this report, future studies will be required to determine what compounds within GSE are responsible for these effects, possibly being gallic acid. Furthermore, future studies will be required to evalute if HDACis across the board lead to patterned up-regulation of tumor-suppressing miRNAs and epigenetic influences by great burnet root (*Sanguisorba Officinalis*), Babul (*Acacia arabica*), Chinese gallnut (*Melaphis Chinensis*), konaberry extract (*Coffea arabica*) and Uva Ursi (*Arctostaphylos uva ursi*).

Table VII. *Similar patterned miRNA changes in GSE and TSA-treated HeLa cells*.

OVERLAPPING miRNA up-regulation by TSA and GSE				
	Fold up-regulation *vs*. Control		Fold up-regulation *vs*. Control	
Transcript ID	GSE	*p*-Value	TSA	*p*-Value
hsa-let-7d-3p	3.38	0.005	2.98	0.027
hsa-miR-23b-5p	6.70	0.049	7.71	0.009
hsa-miR-27b-5p	6.32	0.038	5.98	0.042
hsa-miR-1180-3p	2.37	0.045	3.77	0.004
hsa-miR-6880-5p	6.43	0.016	3.55	0.046
hsa-mir-943	2.24	0.035	2.48	0.048

Conflicts of Interest

The Authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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