



# Profound Inhibitory and Apoptotic Effects of Histone Deacetylase Inhibitor Valproic Acid on Different Cancers

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## Abstract

**Objectives:** Histone acetylation is determined by a balance between the activities of the enzymes that are involved in the histone modifications, including histone deacetylases (HDACs) and histone acetyltransferases which affect gene expression and play a significant role in carcinogens. Valproic acid (VPA) belongs to the HDAC inhibitor family which inhibits HDAC activity and regulate biological events such as apoptosis in various cancers. The current review summarized various pathways by which VPA affects different types of cancers.

**Methods:** For this review, an online search of different sources such as ISI, PubMed, and Scopus resulted in finding the articles correlated with mechanisms and pathways of VPA in different cancers.

**Results:** Based on these results, VPA may be a suitable agent and a good candidate for cancer treatment with multiple mechanisms of apoptosis induction.

**Conclusions:** Overall, VPA can protect against cancer by regulating histone modification and tumor suppressor gene reactivation.

**Keywords:** Valproic acid, Molecular mechanism, Cancer

## Introduction

Nucleosome is the basic unit of the chromatin in the eukaryotic cells, which includes a core histone octamer and the associated DNA chain (1). Chromatin organization affects cellular functions such as translation, transcription, DNA repair, and chromosome segregation (2). In addition, histone proteins are considered as the targets for post-translational modifications such as methylation, acetylation, and phosphorylation (3) all of which play epigenetic regulatory roles including transcription, elongation, and gene silencing (4). The balance between the activities of the two groups of enzymes determines the acetylation level of chromatin (5). The activity of these enzymes also identifies the fate of transcriptional activation (3). Histone deacetylases (HDACs) are the most important target of histone deacetylase inhibitors (HDACIs) as well (6). Histone deacetylation epigenetically affects the development of malignancies and pathogenesis. In vitro evaluation shows that a number of HDACIs can induce cancer cell differentiation, cell apoptosis, and cell growth inhibition in several cancers such as melanoma, leukemia, ovarian, breast, prostate, lung, and colon cancers (7). HDACI compounds contain several chemical structural classes. HDACI valproic acid (VPA) belongs to a short-chain fatty acid family which is administrated as an anticonvulsant agent. Further, experimental

studies indicate that this agent inhibits HDAC activity and thus resulting in apoptosis induction in the selected hematologic and solid tumors.

## Histone Deacetylase Inhibitors

HDACIs are regarded as the significant inducers of cell growth inhibition, cell cycle arrest, and cell apoptosis with different molecular mechanisms (Figure 1). These compounds contain several classes including short-chain fatty acids, benzamides, cyclic tetrapeptides, cyclic peptides, and hydroxamic acids based on general chemical structure (Table 1) and their homologies to the yeast HDACs (Table 2). Mammalian HDACs are divided into four classes (15-17). Clinically, VPA is administrated as an anticonvulsant drug and acts as an HDACI, leading to the histone acetylation which opens the chromatin structure and reactivates the gene expression. The reactivation of tumor suppressor genes can induce apoptosis in solid and hematologic tumors (18).

## Epigenetic and Biological Mechanisms of Histone Deacetylase and HDACIs

In the mammalian cells, multiple molecular pathways are involved in chromatin structure alternation, histone modification, and DNA methylation (Figure 2). Several

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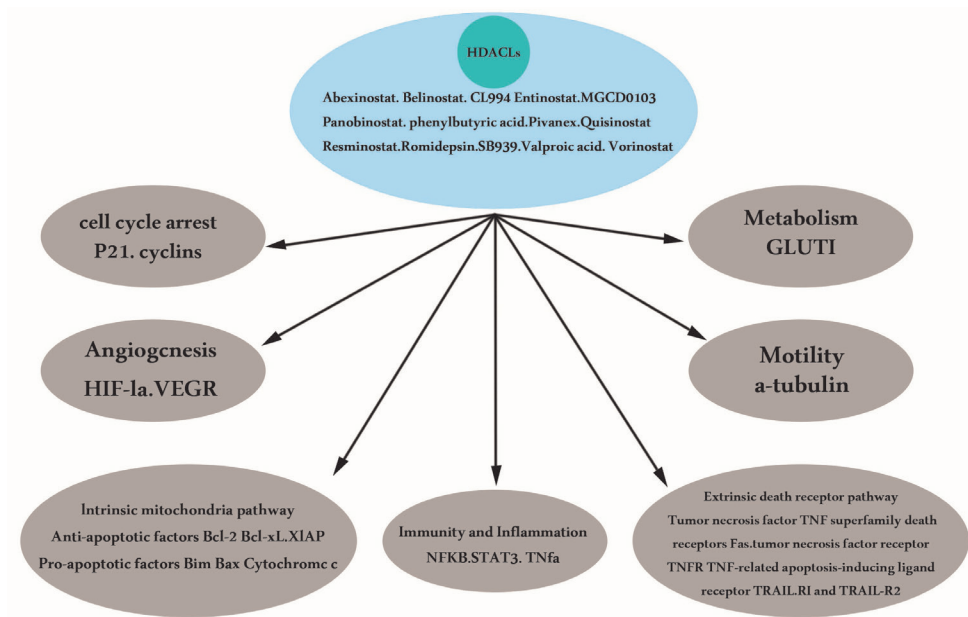


Figure1. Multiple Mechanisms of Histone Deacetylase Inhibitors.

studies indicated that DNA hypermethylation of tumor suppressor genes is accompanied by tumorigenesis. Besides, the hypermethylation of CpG island is one of the most integrated changes in histone modifications, including histone H3 lysine 9 methylation, histone H3 and H4 deacetylation, and histone H4 sumoylation, which

collectively leads to a silenced state of tumor suppressor genes (19).

HDACIs restore histone acetylation which reduces the positive charge of histone proteins, and the affinity of the histone for the DNA chain leads to an open chromatin structure and gene reactivation (Figure 3). In addition

Table 1. Classification and Chemical Structure of HDACIs

Groups	Compounds	Chemical Structure
Hydroxamic acids	TSA, Suberoylanilide bishydroxamide, M-carboxycinnamic acid bishydroxamide, Scriptaid, Pyroxamide oxamflatin	<p>Hydroxamic acids</p>
Short-chain fatty acids	Butyrate, phenylbutyrate, Valproic acid	<p>Short-chain fatty acids</p>
Cyclic tetrapeptides/epoxides	Trapoxin, HC-toxin, Chlamydocin, Depudesin, Apicidine, Depsipeptide	<p>Cyclic tetrapeptides/epoxides</p>
Benzamides	N-acetyldinaline (CI-994), MS-275	<p>Benzamides</p>

Note. HDACIs: Histone deacetylase inhibitors.

**Table 2.** Classification and Cellular Localization of HDACs

Classification and Location of HDACs in Mammals			Location
Classification			
Class I	Zn <sup>2+</sup> -dependent	HDAC1 HDAC2 HDAC3	Cell nucleus Cell nucleus Cell nucleus (rarely in cytoplasm)
Class IIa	Zn <sup>2+</sup> -dependent	HDAC 4 HDAC5 HDAC7 HDAC8 HDAC9	Cell nucleus and cytoplasm Cell nucleus
Class IIb	Zn <sup>2+</sup> -dependent	HDAC5 HDAC6 HDAC10	Cytoplasm Cytoplasm Nucleus
Class IV	Zn <sup>2+</sup> -dependent	HDAC9 HDAC11	Cell nucleus Cell nucleus and cytoplasm

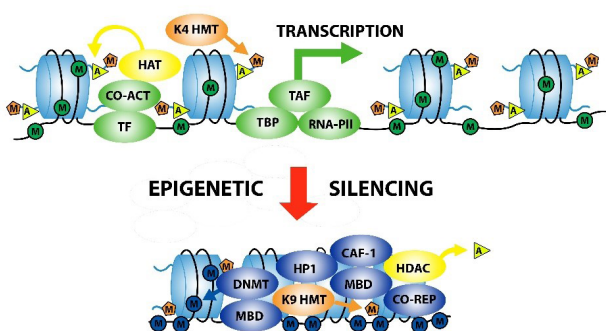
Note. HDACs: Histone deacetylase inhibitors.

to the histone proteins, non-histone proteins could be the substrates of HDACs. This substrate includes DNA-binding proteins, signal transduction molecules, transcription factors, and chaperones (20).

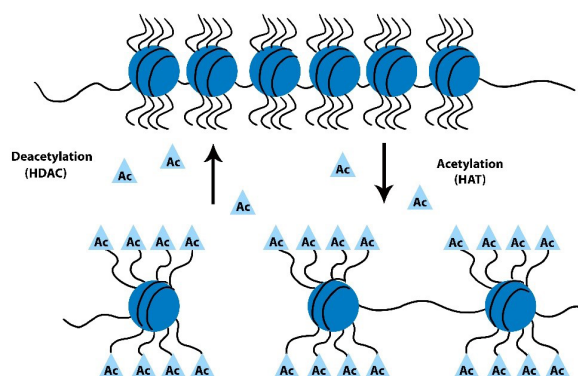
The cells demonstrate different behaviors to HDACs such as transcriptional and non-transcriptional responses. HDACs promote the acetylation of nonhistone and histones proteins through HDAC inhibition. Histones modification can increase or decrease gene transcription (Figure 4). Similarly, they affect the cellular function by multiple biological pathways such as necrosis, apoptosis, cell-cycle arrest, differentiation, migration, and autophagy (21).

**Mechanism of Valproic Acid Signaling Pathways and Targets**

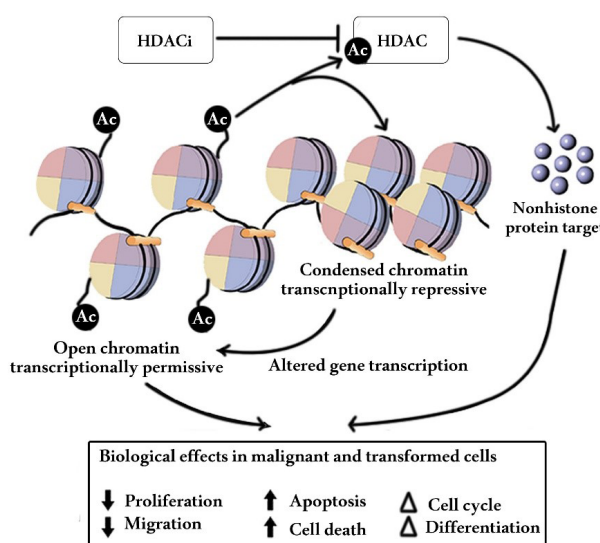
VPA and its analogs target and inhibit class I and II HDACs except for HDAC6, HDAC9, and HDAC10. These compounds induce the acetylation of core histones H3 and H4 including 2M2P (2-methyl-pentenoic acid), 2M2PP (2-methyl-2n-propylpentanoic acid), 2EH (2-ethylhexanoic acid), 4PA (4-pentenoic acid), and



**Figure 2.** Epigenetic Silencing Pathways. The figure shows several pathways that occur in the tumor suppressor genes and lead to silenced genes in tumor cells. In addition, the open chromatin is transcriptionally active (blue cylinders) and the tightly packed chromatin is transcriptionally silenced which is displayed at the bottom.



**Figure 3.** Histone Acetylation and Gene Expression Regulation. The upper panel indicates the structure of the nucleosome. Furthermore, the lower panel shows the effect of the acetylation of histones (blue triangles) by histone acetyltransferase enzyme which repels the histone away from DNA and exposes histone-free DNA for gene expression.



**Figure 4.** Histone deacetylase (HDAC) inhibitors inhibit HDAC Enzymes Resulting in the Acetylation of Nonhistone and Histones Proteins.

valproic acid (22). Figure 5 illustrates chemical structures, as well as the targets of VPA and its analogs.

VPA affects the cell growth and differentiation of tumor cells by HDAC inhibition.

It further exerts its effect through histone acetylation and deacetylation, mainly histones H3 and H4 (Figure 6). In addition to the inhibitory effect on HDACs, the other pathways include kinases GSK3 $\beta$  and ERK (23).

Experimental studies reveal that VPA can modify chromatin structure in MCF-7 breast cancer cells through the repression of SMC 1 to 5, DNMT-1, and HP1.

This alteration in chromatin structure increases the sensitivity of the DNA strand to the nucleases, which results in the re-activation of gene expression. Furthermore, VPA potentiates cell apoptosis and DNA damage induced by cytotoxic components that require access to the DNA strand for their activities (24). An in vitro study demonstrated that VPA can inhibit cell proliferation in several breast cancer cell lines including ER-negative and HER2-negative MDA-MB-231 cells, HER2-negative and ER-positive MCF7 cells, HER2-negative and ER-positive BT474 cells, as well as HER2-overexpressing and ER-negative SKBR3 cells. In breast cancer, VPA can increase HER2-expression resulting in the inhibition of cell proliferation and can induce cell cycle arrest via p21 up-regulation (25). Additionally, it is an anticonvulsant

drug that activates transcription from various promoter regions and activates Wnt-dependent expression while not inhibiting in vivo GSK-3 $\beta$ . Further research reported that VPA activates Wnt-dependent expression via HDAC inhibition resulting in the decreased expression of Tcf/Lef and the increased expression of  $\beta$ -catenin (26).

### Mechanism of VPA and Ovarian Cancer

According to experimental studies, VPA can significantly inhibit the proliferation of SKOV3 ovarian cancer cells which is related to the cell cycle arrest and cell apoptosis induction. This effect is associated with the increased G1-phase and decreased S-phase of the cell cycle without significant changes in M-phase and G2-phase.

The cell growth inhibition of SKOV3 ovarian cancer cells leads to a reduction in the S-phase of the cell cycle because of blocking at the G1-phase checkpoint of the cycle.

It is also reported that VPA can down-regulate vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9) in addition to up-regulating E-cadherin expression and interrupting metastasis and tumor angiogenesis through VEGF protein down-regulation, along with E-cadherin and MMP-9 protein up-regulation (27). Other researchers demonstrated that VPA can suppress cell growth in nine human ovarian cancer cell lines such as SK-OV-3, IGROV1, TOV-21G, TOV-112D, OVCA420, OV-90, OVCA432, OVCA433, and OVCA429 (28). One of the molecular mechanisms through which VPA inhibits cell proliferation includes the up-regulation of p27KIP1 and p21WAF1, two proteins that block the G1-phase, and down-regulate several cell cycle-related and antiapoptotic proteins such as cyclin D1, cyclin D2, and Bcl-2 (29). Besides, VPA can inhibit ovarian cancer SK-OV-3 cell proliferation in the mice after five weeks of treatment and suppress the cell growth of xenograft tumors with a significant up-regulation of p21 (28).

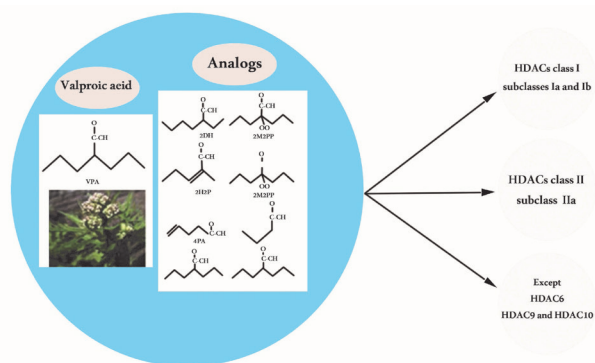


Figure 5. Chemical Structures of Valproic Acid, Along With its Analogs and Substrates.

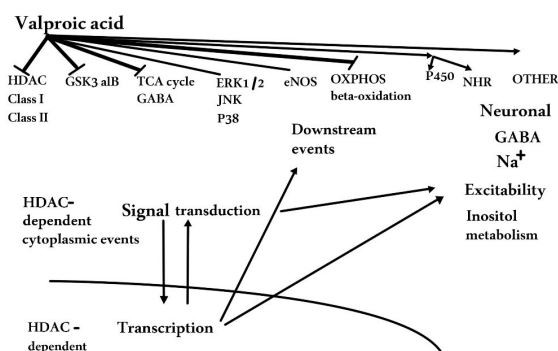


Figure 6. Multiple Molecular Mechanisms of Valproic Acid and Their Interactions.

### Mechanism of VPA and Uterine Cancer

Similarly, recent experimental works have demonstrated that VPA can inhibit cell growth in various endometrial cancer cells such as Hec59, Hec-1B, Ishikawa, KLE, AN3CA, and RL95-2 with a dose-dependent fashion (30). In these cell lines, VPA up-regulates p27KIP1 and p21WAF1 expressions and increases the protein levels of both expressions (31). Based on the reports of another study, VPA enhances p21WAF1 expression resulting in acetylated histones H3 and H4 accumulation (32) while decreasing the transcription level of cyclin D1 and D2. Likewise, it was shown that VPA increases p21WAF1 gene expression whereas it reduces D cyclins gene expression through the modulation of the activity of pRb/E2F (33). In the cervical cancer Hela cells, VPA induces apoptosis and inhibits proliferation through tumor suppressor gene Notch1 up-regulation, along with the up-regulation of

other cancer-associated genes such as PCNA, p63, and p21 (34). Besides, this medication inhibits the activities of nuclear and cytosolic HDACs in this cell line (22), the effect of which is time-dependent. In addition, it induces G2/M phase arrest at 10 mM concentration of VPA in HeLa cervical cancer cells, a low dose of the agent induces a G1-phase arrest in this cell line (35). Further, the apoptotic effect of VPA is associated with caspase-3, -8, and -9 activation (36). It also triggers the loss of MMP in cervical cancer HeLa cells by a dose and time-dependent fashion. Besides, VPA can activate cell death receptor and the mitochondrial pathway, all of which are necessary for the apoptosis of HeLa cells which are treated with VPA. Recent evidence has also revealed that TNF- $\alpha$  synergistically increases cell death in tumor HeLa cells that are treated with VPA. Therefore, the major mechanism of VPA-treated HeLa cell death is mediated through apoptosis rather than other mechanisms such as necrotic cell death pathway (37).

#### **Mechanism of VPA and Prostate Cancer**

VPA induces apoptosis through p21/Waf1/CIP1 up-regulation and *TMPRSS2-ERG* repression in prostate cancer cells (38,39). The findings of previous research confirmed that aberrant *ERG* expression plays a significant role in prostate cancer and cancer cell migration and that the overexpression of *ERG* is accompanied by the prognosis of this cancer. It was also reported that VPA induces changes in the chromatin remodeling in prostate cancer cells (40), increases the histone acetylation of H3 and p21, p27, and CK18 expressions while decreasing Ki-67, cyclin D1, and androgen receptor expression in prostate cancer cells. Other researchers found that a low concentration of VPA decreases angiogenesis whereas it increases apoptosis in the prostate cancer xenografts (41-43). VPA has a significant effect on reducing the HDAC activity in LNCaP cells at a low concentration and induces apoptosis by caspase-3 activity and DNA fragmentation (44). Likewise, this drug can suppress cell proliferation, overexpress the E-cadherin gene, and finally, increase androgen receptor levels in prostate cancer cells (45). It can induce the neuro-endocrine transdifferentiation in androgen-independent PC3 cells as well (46). Furthermore, it was reported that prostate glands include a small population of neuroendocrine cells in the epithelial compartment (47). Moreover, VPA can up-regulate various androgen metabolism genes and increase dihydrotestosterone catabolism (48). Based on the findings of another research, a low concentration of VPA has an inhibitory effect on LNCaP cells in that it contributes to differentiation status and suppresses differentiated LNCaP cells (49).

#### **Mechanism of VPA and Gastric Cancer**

Several researchers also approved the antiproliferative effect of VPA on OCUM-2MD3 gastric cancer cell

line. For example, it was revealed that VPA increases the acetylation of histone H3 in this cell line and leads to cell growth suppression via p21WAF1 induction (50). In addition, the acetylation of histone H3 by the overexpression of p21WAF1 suggests that VPA can induce cancer cell differentiation (22). Likewise, VPA can alter the expression level of cyclin D1 and p27. Further, P27 and P21WAF1, as cyclin-dependent kinase inhibitors, decrease kinase activity by binding to cyclin-dependent kinase complexes and thus reduce kinase activity (29). In gastric cancer, this drug can also induce apoptosis via extrinsic and the intrinsic pathways, as well as c-Myc, Cyclin A down-regulation, and P21 (Waf/cip1) up-regulation to induce cell cycle arrest in G1-phase (35). Other studies indicated that VPA can significantly inhibit gastric carcinoma cell growth including SGC-7901, BGC-823, and HGC-27 cell lines in G1-phase by the activation of caspases (3, 8, 9). The molecular pathway of G1-phase cell cycle arrest is related to Mad1 and P21 up-regulation in addition to c-Myc and cyclin A down-regulation (35).

#### **Mechanism of VPA and Hepatocellular Carcinoma**

The anti-tumor activity of VPA is reported in many different cancers (51). In HTB-52 hepatocellular carcinoma (HCC) cells, VPA induces histone H4 acetylation and suppresses HDAC4 by which inhibits cell growth inhibition and induces cell cycle arrest and apoptosis (54). A similar effect is demonstrated in the other HCC cell lines (55). Notch signaling is known as a diagnostic marker in HCC and the activation of this mechanism is observed in human HCC. The high expression of Notch1, Notch3, and Notch4 is reported in the most HCCs as well (54). Similarly, a high frequency of Notch1 and Notch3 is detected in HCC tissues. As a critical factor in HCC, HBV X protein (HBx) up-regulates the Notch signaling pathway (55). Accumulating evidence shows that the activation of this pathway plays an oncogenic role in HCC (56). Furthermore, VPA suppresses Notch1 and Notch target gene HES1 expression and reverses the cell proliferation stimulated by Notch1 activation. Thus, VPA can inhibit HCC cell proliferation through Notch signaling inhibition. Besides, the inhibitory effect of VPA, as a HDAC1 inhibitor, is shown in HCC BEL-7402, SMMC-7721, and HepG2 cell lines (57). Likewise, the significant up-regulation of cyclins A, D1, and E is reported in tumor tissues, which can be inhibited by P2. The abnormal expression of these cyclins is associated with tumorigenesis and cancer progression (58). On the other hand, VPA up-regulates the protein expression of caspases (3 & 9), leading to the apoptosis of HCC HepG 2 cell line. We previously reported that VPA can inhibit cell growth and induce apoptosis in HCC HepG 2 cell line (59).

#### **Mechanism of VPA and Cholangiocarcinoma**

VPA has a potent anticancer effect against various tumor

tissues including colon, prostate, bladder, breast, thyroid, liver, and colon cancers (18). It affects the behavior of various cancers by different mechanisms such as cell differentiation, metastasis, angiogenesis, cell apoptosis, and cell cycle arrest (60). Moreover, it is indicated that VPA inhibits the proliferation of cholangiocarcinoma (CCA) QBC939, TFK-1, CCLP1, and QBC939 cells. Additionally, it can induce the terminal differentiation of CCA TFK-1 cells resulting in cell growth inhibition. Based on the findings of an in vitro study, the inhibitory effect of VPA on QBC939 and TFK-1 cells is associated with cell apoptosis and cell cycle arrest. VPA treatment suppresses the growth of TFK-1 cells at the G2/M phase (61). Besides, it increases the inhibitory effect of 5-fluorouracil on CCA HuCCT1 cell line (62). We previously reported the apoptotic and inhibitory effect of this compound on the HT-29 colon cancer cell line as well (14).

### Mechanism of VPA and Pancreatic Cancer

Several in vitro studies also represented that VPA modulates the behavior and biology of different cancers by inhibiting cell proliferation, angiogenesis, metastasis, as well as inducing cell differentiation and cell apoptosis. According to some recent studies, VPA has an inhibitory effect on pancreatic stellate cell proliferation (63,64). It also exerts anti-tumor effects by NKG2DL up-regulation such as UL16-binding proteins and MICA/B in several cancers including myeloma, HCC, and myeloid leukemia. These anti-tumor effects are linked to the activation of different molecular mechanisms in various cancers (65).

### In Vitro Effect of VPA

VPA induces cell growth inhibition, cell differentiation, cell cycle arrest, and cell apoptosis in a medulloblastoma cell line in a time- and dose-dependent fashion (66). Similarly, it enhances the radiosensitivity of human brain tumor cell lines U251 and SF539 which correlates with the histone hyperacetylation (67). In addition, VPA affects human leukemia cells by stimulating caspase-independent and caspase-dependent pathways. It also induces apoptotic changes such as phosphatidylserine externalization, DNA fragmentation, caspases (3, 8, and 9) activation in MV411 cells (68). Likewise, VPA can increase the E-cadherin expression while decreasing VEGF and MMP-9 in ovarian SKOV3 cells (69). In gastric cancer OCUM-2MD3 cell lines, VPA increases acetyl- $\alpha$ -tubulin, acetyl-histone H3, and p21WAF1 levels accompanied by the up-regulation of caspases (3 & 9), p27, cyclin D1, and bcl-2 down-regulation (70).

### Conclusions

Overall, the data summarized in the current review demonstrated that VPA exerts apoptotic and inhibitory effects on various cancers through the inhibition of HDACs, which affect the remodeling of chromatin

structure and result in the reactivation of tumor suppressor genes.

### Conflict of Interests

Authors have no conflict of interests.

### Ethical Issues

Not applicable.

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