Effect of valproic acid on histone deacetylase expression in oral cancer (Review)

AHMED S.K. AL-KHAFAJI[1-3], LYDIA M. WANG[4], HAIDAR H. ALABDEI[3] and TRIANTAFILLOS LILOGLOU[5]

[1]Department of Molecular and Clinical Cancer Medicine, University of Liverpool, Liverpool L7 8TX, UK; [2]Department of Biology, College of Science, University of Baghdad, Baghdad 10071; [3]College of Medicine, University of Warith Al-Anbiyaa, Karbala 56001, Iraq; [4]Centre for Haemato-Oncology, Barts Cancer Institute, Queen Mary University of London, London EC1M 6BQ; [5]Cardiorespiratory Research Centre, Medical School, Faculty of Health, Social Care and Medicine, Edge Hill University, Ormskirk, Lancashire L39 4QP, UK

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Abstract. Oral squamous cell carcinoma (OSCC) is a frequent human malignancy that demonstrates a range of genetic and epigenetic alterations. Histone deacetylases (HDACs) are key epigenetic regulators of cell-cycle progression, differentiation and apoptosis and their dysregulation is implicated in cancer development. HDACs are promising targets for anticancer therapy through the utilisation of HDAC inhibitors (HDACis). OSCC cells have been shown to have low levels of histone acetylation, suggesting that HDACis may produce beneficial effects in patients with OSCC. Valproic acid (VPA) is a class I and IIa HDACi and, therefore, may be useful in anticancer therapy. VPA has been reported as a chemo-preventive epigenetic agent in individuals with high-risk oral dysplasia (OD) and thus associated with a reduced risk of HNSCC. It is hypothesised that HDAC inhibition by VPA triggers a change in the expression levels of different HDAC family gene-members. The present review summarises the current literature on HDAC expression changes in response to VPA in oral cancer patients and *in vitro* studies in an effort to better understand the potential epigenetic impact of VPA treatment. The present review outlined the need for exploring supportive evidence of the chemo-preventive role played by VPA-based epigenetic modification in treating oral pre-cancerous lesions and, thus, providing a novel tolerable chemotherapeutic strategy for patients with oral cancer.

Correspondence to: Dr Triantafillos Liloglou, Cardiorespiratory Research Centre, Medical School, Faculty of Health, Social Care and Medicine, Edge Hill University, St Helens Road, Ormskirk, Lancashire L39 4QP, UK
E-mail: liloglol@edgehill.ac.uk

Abbreviations: HNSCC, head and neck squamous cell carcinoma; OD, oral dysplasia; MT, malignant transformation; HAT, histone acetyltransferase; HDAC, histone deacetylase; HDACi, histone deacetylase inhibitor; VPA, valproic acid; TRAIL, TNF-related apoptosis-inducing ligand; GEM, gemcitabine; UC, urothelial carcinoma

Key words: valproic acid, histone deacetylase expression, oral cancer, histone deacetylase inhibitors, preneoplasia

Contents

1. Introduction

Head and neck cancer is the eighth most common form of neoplasia and the fifteenth most common cause of cancer-related deaths in the UK (1). Oral cancer is the most common form of head and neck cancer and most frequently is of squamous histology (oral squamous cell carcinomas-OSCC). OSCC includes cancers of the oral cavity including tongue throat, lips and gums (2). Oral cancers are often diagnosed in the advance stages of the disease, consequently, decreasing the probability of curative treatment (3). A frequent precursor to oral cancer is oral dysplasia (OD), which presents as white or red lesions (leukoplakia or erythroplakia, respectively). However, ODs do not always undergo malignant transformation (MT) and can remain benign. Leukoplakia is the most prevalent and the rate of MT is 5-17% (4,5). Tobacco and alcohol use are well known risk factors of oropharyngeal squamous cell carcinoma (OPSCC) (6-9), and are also primary risk factors of developing OD (5,10).

Currently, the major challenge in the clinical management of ODs is the accurate prediction of MT. The risk of MT is graded as high/low through clinical observation. However, this

is subjective and often results in misdiagnosis (11). Surgical removal of precancerous lesions can be performed as a preventative treatment, but it cannot guarantee a lack of recurrence and can cause long-term morbidity for patients, such as dysarthria and dysphagia (11,12). The Liverpool Management Algorithm, provides OD management advice based on the available evidence (13). However, accurate prediction of MT remains elusive, pointing to the potential application of chemoprevention in OD patients.

2. Histone acetylation

Cancer development is associated with genetic mutations (14), as well as epigenetic changes, which can alter chromatin structure (15). Histone tail acetylation is an important epigenetic change, which is involved in the regulation of gene expression (14,16). This is controlled by two enzymatic groups; histone acetyltransferases (HATs) and histone deacetylases (HDACs) (17,18). HATs transfer an acetyl group to the lysine residue of the N-terminal of histones (19). This results in a relaxed chromatin structure and expression activation. HDACs catalyse the hydrolytic removal of acetyl, causing chromatin condensation and transcriptional silencing (14,19). HDACs also deacetylate non-histone proteins involved in the regulation of cell-cycle progression, differentiation and apoptosis (14). An imbalance between HATs and HDACs activity is implicated in a number of human diseases, such as neurodegenerative (20) and cardiovascular diseases (21), and cancer (22,23).

HDACs are divided into four classes: Class I (HDAC1, HDAC2, HDAC3, HDAC8); Class II, which is subdivided into Class IIa (HDAC4, HDAC5, HDAC7, HDAC9) and Class IIb (HDAC6, HDAC10); and Class IV (HDAC11) (Table I) (16). Class I, II and IV share a common mechanism that requires zinc for their enzymatic activity. Class III (sirtuins, SIRT1-7) are dependent on NAD^+ rather than zinc. HDACs demonstrate a remarkable variability regarding the processed RNA transcript splice variants and consequent protein isoforms (Table I) (https://www.ncbi.nlm.nih.gov/ and https://www.rcsb.org/). This diversity creates complex substrate specificity of HDACs and, therefore, produces a diverse range of functions (18). Furthermore, in addition to acetylation, HDACs can undergo alternative post-translational modifications including, methylation, phosphorylation and ubiquitination, which can alter the enzymatic activity of HDACs in different ways. For example, phosphorylation of HDAC1 increases its activity and phosphorylation of Class IIa HDACs determines their cellular localisation (16). Overall, the different variable factors mentioned, produce huge functional variability of HDACs and, therefore, allow many possible opportunities for interference with human diseases.

3. HDAC function

Class I HDACs are ubiquitously expressed and are involved in cell proliferation and survival (24). HDAC1, HDAC2 and HDAC3 have repressive functions, for example, HDAC1 and HDAC2 repress p21 and p57, which are involved in the progression of the cell cycle (25). Class II have more tissue-specific functions than other HDACs (26). They freely shuttle between the nucleus and cytoplasm, suggesting their interaction with non-histone proteins. Localisation is determined by phosphorylation, which also regulates transcriptional repression capacity (24). For example, HDAC9 represses myocyte enhancer factor-2 until the enzyme receives a signal to be transported to the cytoplasm. Class IIb HDACs are structurally different to Class IIa, due to a second catalytic domain (16). HDAC6 has a role in the clearance of misfolded proteins, which makes it an important target for Alzheimer's disease (20). Currently, little is known about the function of Class IV HDACs.

4. HDAC inhibition and cancer

Acetylation is involved in the regulation of important oncogenic mechanisms (24). Therefore, due to frequent increased HDAC expression and activity in cancer, tumour formation is promoted (14). However, the expression pattern can differ between tumour types; high HDAC8 expression has been associated with poor prognosis of neuroblastoma patients and HDAC1, HDAC2 and HDAC6 have been shown to be upregulated in HNSCC (27,28). HDACs play a role in the silencing of tumour suppressor genes, therefore, an increase in their activity would exaggerate this function. Ultimately, this will result in effects, such as cell-cycle persistence and apoptosis reduction.

HDACs are promising targets for anti-cancer therapy, specifically through utilisation of HDAC inhibitors (HDACis) (24). Heterogeneity of HDAC expression in tumour types, however, poses a challenge (29). HNSCC cells, specifically, have been shown to have low levels of histone acetylation, suggesting that HDACis may produce beneficial effects in patients (23,28). There are five classes of HDACi; hydroxamic acids, short-chain fatty acids, benzamides, cyclic tetrapeptides and sirtuin inhibitors (24). Among these are pan-HDACis, which inhibit all HDAC classes, while others exert specificity against certain HDAC classes (29). HDACis that are currently clinically approved include, Vorinostat (SAHA), Belinostat (PXD101), Panobinostat (LBH589), Romidepsin (FK228), Chidamide (CS055/HBI-8000), while there are more currently in clinical trials (18).

5. Valproic acid and oral cancer therapy

The short-chain fatty acid, valproic acid (VPA), is currently under investigation in the treatment of cancer (Fig. 1) (23,30-32). VPA is a well-established treatment for epilepsy and other neurological diseases (33). VPA is described to have various mechanisms of action contributing to its anti-epileptic effects, however, these pathways are yet to be fully understood. Suggested mechanisms include; inhibition of voltage-gated sodium channels resulting in blockade of abnormal electrical impulses responsible for seizures and interference with gamma-aminobutyric acid (GABA) signalling through inhibition of GABA transaminase or promotion of GABA synthesis, again, preventing occurrence of seizures. More recently, it was reported that VPA is a Class I and IIa HDACi, therefore, may be useful in anti-cancer therapy (34,35). Binding studies suggest that VPA exerts its HDAC inhibitory function through blockade of substrate binding to the catalytic centre of HDAC enzymes (36). It is thought that this is via interaction of the carboxyl group of VPA with Zn and other

Table I. HDAC classification highlighting the high variability of HDACs due to splice variance.

HDAC class	Co-factor	No. of exons	No. of transcript variants	Chromosome location
Class I	Zn^{2+}			
HDAC1		14	1	1p35.2-p35.1
HDAC2		14	3	6q21
HDAC3		15	10	5q31.3
HDAC8		11	7	Xq13.1
Class IIa	Zn^{2+}			
HDAC4		26	5	2q37.3
HDAC5		26	3	17q21.31
HDAC7		25	6	12q13.11
HDAC9		10	40	7p21.1
Class IIb	Zn^{2+}			
HDAC6		28	11	Xp11.23
HDAC10		20	2	22q13.33
Class III	NAD^{+}			
SIRT1		9	3	10q21.3
SIRT2		14	5	19q13.2
SIRT3		6	33	11p15.5
SIRT4		3	4	12q24.23-q24.31
SIRT5		8	26	6p23
SIRT6		7	9	19p13.3
SIRT7		10	1	17q25.3
Class IV	Zn^{2+}			
HDAC11		10	3	3p25.1

HDAC, histone deacetylase; SIRT, sirtuins.

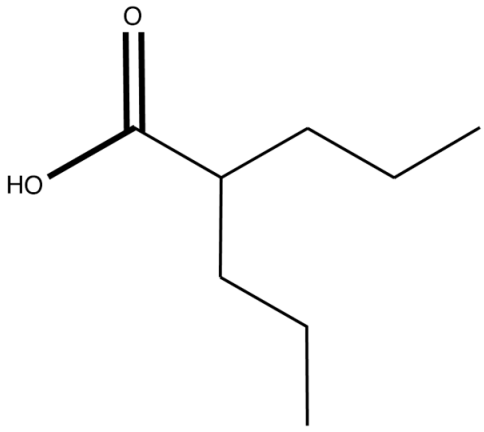

Figure 1. Valproic acid chemical structure. The carboxylic acid group that is considered to interact with histone deacetylase catalytic sites is shown in bold.

residues of HDAC active sites (Fig. 1) (37). A large-scale study investigating long-term VPA treatment for psychiatric diseases in US veterans, reported a significant association of VPA with a reduced risk of HNSCC (38). This same result was not observed for other tumour types, suggesting that VPA may not be useful for all cancers. Consequently, VPA presents as an encouraging treatment for HNSCC specifically.

Potent *in vitro* and *in vivo* growth inhibition has been reported following VPA treatment (30,39,40). VPA can inhibit the growth of HNSCC cells through upregulation of p21 and induction of G0/G1 arrest (30), while similar results were found in breast cancer cells (31). VPA interferes with the self-renewal of HNSCC cancer stem cells and suppresses expression of stem cell markers (39).

In addition to VPA use as a single agent, favourable results are shown for its use in combination treatment of HNSCC patients (32,38-40). VPA is shown to potentiate the antitumour effect of cisplatin and cetuximab in HNSCC xenografts (41). VPA may, therefore, sensitise cancer cells to chemotherapeutics, improving their efficacy and subsequently reducing the necessary dose, resulting in lower toxicity and resistance.

The ongoing SAVER clinical trial investigates VPA as a chemo-preventive epigenetic agent in individuals with high-risk OD (42). This randomised, double-blind, placebo-controlled trial measures the histological and clinical response rate of OD to VPA. Therefore, determining its use as a preventative treatment for MT of high-risk OD. A previous study has reported HDAC2 upregulation in pre-cancerous ODs (43), further supporting this hypothesis. A mechanistic study is conducted in parallel to SAVER to define the mechanism of action of VPA in HNSCC cells.

Questions surrounding the cellular responses and how pathways are affected by HDAC inhibition remain unanswered. In

particular, the way HDACis influences the expression of their target genes is not fully elucidated. It is possible that by-pass and feedback loops may be in play, so that when cells are exposed to HDACis, changes in expression levels of HDACs may be triggered (44). In addition, the expression levels of HDACs could potentially be used as markers of response to HDACis in patients (45). Therefore, understanding the specific expression patterns of HDACs in cancers before and after HDACi treatment is important.

Valproic acid (VPA) has been considered a good candidate for anticancer therapy. A reasonable option may be to employ it as monotherapy (46) or in combination (32,47) with other chemotherapeutic agents in recurrent and/or metastatic squamous cell carcinoma of Head and Neck (SCCHN) trials. Two studies reported changes in HDAC expression with VPA in combination treatment (48,49). A reduction in HDAC4 protein levels was found in a head and neck cancer cell line when cells were treated with VPA in combination with the tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), compared to TRAIL treatment alone (49).

HDAC1 mRNA downregulation was reported in a human cholangiocarcinoma cell line when VPA was used in combination with gemcitabine (GEM), compared to GEM as a single agent (48). These findings indicate that VPA may sensitise cells to other treatments, therefore, may be useful for combination therapy.

6. HNSCC prognosis

In recent years, the prognosis and survival of HNSCC have seen a minor improvement, however, the 5-year overall survival rate remains low, at approximately 40-60% (5,50). Early diagnosis of HNSCC is key to ensuring the best possible outcome for patients and improves survival to 80% (28,51). However, currently, there is a lack of prognostic and predictive markers of HNSCC, which restricts early diagnosis. Therefore, the majority of HNSCC cases are diagnosed in the later stages of the disease and more aggressive treatment is necessary (52).

ODs are a common precursor to oral HNSCCs (13). However, the occurrence of these ODs does not necessarily equate to cancer. There is a potential for the lesions to undergo MT, with factors, such as tobacco use, increasing the probability (5). Therefore, prediction and prevention of the transformation of precancerous ODs are extremely important to increase the survival of HNSCC. Currently, the methods to do this are surgery or the prediction of cancerous lesions by observation. However, surgery often leads to long-term issues for patients and misdiagnosis is common with observation (53). Therefore, there is an unmet clinical need for better prevention or prediction of MT to reduce oral cancer cases.

7. Cancer chemoprevention by HDAC inhibition

Due to developments in research, it is now known that cancer development not only arises due to genetic alterations but can also arise from changes in epigenetic mechanisms as well (29,54). Acetylation is a crucial histone modification that has an important role in chromatin remodelling. Interruptions to the balance of HATs and HDACs, leading to hyper or hypoacetylation of histone and non-histone proteins, has been shown to be implicated in a number of human diseases (21,27). In particular, HDACs involvement with cancer has been highlighted in a number of studies, with results indicating that HDAC expression is increased in certain cancers (19,55,56). This is a significant alteration due to multiple functions of HDACs implicating tumour progression mechanisms. For example, Class I HDACs repress the transcription of the cell-cycle inhibitor, p21 (24). Consequently, if HDACs are overexpressed, this may contribute to the uncontrolled proliferation of cells. Moreover, Chang *et al* reported that HDAC2 expression is upregulated in oral pre-malignant lesions, suggesting that HDACis could be used for chemoprevention in oral cancers (43).

8. VPA modulates HDACs in HNSCC

The recent discovery of HDAC inhibition for cancer treatment has seen the approval of five HDACis for clinical use (24). Compared with traditional anti-cancer therapies, HDACis offer a much-improved toxicity profile, due to minimal effects on normal cells (16). Although clinically manageable, there are still toxicities associated with HDACis, including thrombocytopenia, nausea and vomiting. However, VPA, which is in phase II trials, does not exhibit these side effects and it is known that long-term use is tolerable for patients due to its well-established use as an anti-epileptic (32). Therefore, in addition to the encouraging *in vitro* and *in vivo* evidence, VPA appears to be an attractive anti-cancer agent (30,31,41,57). Furthermore, the epidemiological study by Kang *et al* suggests that VPA treatment is associated with a lower risk of HNSCC development and, therefore, may be a suitable candidate for treatment and/or chemoprevention (38).

Due to the plethora of targets and functions exerted by HDACs, HDACis can act in multiple different ways (44). In addition, the mechanism of action by which HDACis act differs according to the cancer being treated and the inhibitor being used. Therefore, there is still much unknown about the biological mechanisms of HDACis (22,58). Understanding the precise mechanisms of action is key to elucidating which cancers are best treated by HDACis and the specificity of which inhibitor for which tumour type. For example, VPA was found to be effective in reducing the incidence of HNSCC, but not the incidence of lung cancer (38). Therefore, understanding why this happens will allow improved treatment strategies.

The present review aimed to explicate if HDACis alter the expression pattern of HDACs and, in particular, whether VPA could alters the expression of HDACs in oral cancer. From the studies presented, it is clear that HDACis can regulate the expression levels of HDACs both at the mRNA and protein level. However, the reported changes vary between studies. This is likely due to the involvement of different HDACs per cancer, which may alter the outcomes produced by each HDACi (29). The HDACi, apicidin, has been investigated in three different cancers across three studies (59-61). The variation in results demonstrates the theory that different HDACs are involved between cancers, which, therefore, alters the inhibitory effects of individual HDACs. In addition to differing levels of upregulation, HDACs can also harbour mutations, which vary greatly in frequency between cancers (62). For example, lung cancer and melanoma have a high percentage of mutations in

all HDACs, whereas, ovarian and glioblastoma have very few. These factors, in addition to the different targets of HDACis, may explain the variety of results found here.

The majority of studies reviewed herein report a decrease in HDAC expression following VPA treatment. However, in some cases, an upregulation of HDACs is seen. This may be due to a compensatory mechanism against HDAC inhibition that has been previously described (63). This has been suggested for HDAC1 and HDAC2 where one HDAC is downregulated to enable the upregulation of the other. However, some of the studies reviewed only investigated one HDAC, making it difficult to determine if a compensatory mechanism is in place. In the studies that investigated multiple HDACs, fluctuations of different HDACs expression are observed. In 19i-treated UC cells, a decrease in HDAC7 is reported, whilst there is an increase in HDAC4 (64). This is due to 19i demonstrating preferential inhibition against HDAC4 but not HDAC7. Therefore, the decrease in HDAC7 allows increases in HDAC4 expression to combat the loss of function. Not only does this suggest a compensatory mechanism, but it also implies that HDACs can regulate the expression of one another. In addition, this indicates that HDAC inhibition may alter the *de novo* synthesis of HDACs to counteract the loss of function. These results support the hypothesis that HDAC inhibition triggers feedback for the expression control of HDAC genes.

9. Role of VPA in HNSCC treatment

The limited number of studies available reflects our largely incomplete understanding of the different HDAC expression changes and their functional consequences in the wider spectrum of tumour types. Among the studies reviewed, only five cancer types were investigated more than once, making it difficult to validate results. Therefore, there is a critical need for more research to determine the differential expression of HDACs and how inhibitors affect them in individual cancers, especially in those that lack well-defined prognostic factors that lead to poorer therapeutic management, such as HNSCC (52).

10. Conclusion

HDACs expression has significant clinical impact in oral cancer (65). Although the search conducted here reviewing several studies reported the therapeutic role of VPA in treating oral cancers, only one study has clearly reported that lowering HDAC expression following VPA treatment has induced cellular death in oral cancer (49). In addition, we reviewed few studies that reported its use in combination treatment of HNSCC rather than alone. Nevertheless, the studies have not investigated the inhibitory changes in HDAC expression after VPA was involved in the therapeutic regimen. Another study demonstrated reduced HDAC7 expression in oral cancer after HDACi treatment (59), providing further evidence that HDAC inhibition may have an effect on HDAC expression in oral cancers. Clearly, there is a crucial need for further research into HDAC expression changes in oral cancers treated with HDACis, especially, since reports of the specific efficacy of VPA in this tumour type (38). Therefore, due to the evidence of HDAC upregulation in pre-cancerous lesions and oral cancers, additional efforts should be given to further clarify the changes and the epigenetic landscape caused by VPA treatment.

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ASKAK made substantial contributions to the conception and design of the review and drafted the work. LMW contributed to the acquisition, analysis and interpretation of literature data, and revised the manuscript. HHA contributed to the acquisition of literature data, interpreted the data and revised the manuscript. TL participated in the acquisition and interpretation of literature data and revised the manuscript. All the authors have read and approved the final version of the manuscript for publication. Data authentication is not required.

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Authors' information

Dr Ahmed S.K. Al-Khafaji, ORCID ID: 0000-0002-6802-5816; Miss Lydia M. Wang, ORCID ID: 0009-0004-8072-0418; Dr Haidar H. Alabdei, ORCID ID: 0000-0003-1960-7331; Dr Triantafillos Liloglou, ORCID ID: 0000-0003-0460-1404.

Competing interests

The authors declare that they have no competing interests.

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