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Original article

Differential Expression of HDAC1, HDAC2, HDAC3, HDAC5, HDAC6, HDAC7, and HDAC8 in Ovarian Cancer Cell Lines Compared to Normal Ovarian Epithelial Cells

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Abstract
Diagnosing tumors in a specific manner with small molecules, such as histone deacetylases (HDACs), is instantly necessary. HDAC class II plays a crucial role in regulating the expression of genes through their enrollment by transcription factors during tissue growth and development. Further, HDAC class has distinguished properties that make it differ from the first HDAC class; the size of these proteins is larger than class I, Also, in this class, the catalytic domain is located in the carboxy-terminal half of the protein. This study investigated the expression levels of individual human HDAC class II family members in epithelial ovarian cancer cell lines because they play a certain role in cellular mechanisms, including cell proliferation, cell cycle progression, and apoptosis. Through HDACII, special effects on the stress response, oncogenesis, cell motility, and many other cancer-related signaling networks. HDACs organize impact the pathways of ovarian cancer. Experimental Design: In human ovarian surface epithelial cell line and SKOV-3, OAW42, the relative gene expressions were quantified for screening HDAC1, 2, 3, 5, 6, 7, and 8 mRNA using QRT-PCR and immunocytochemistry performed to study the expression of the mentioned HDACs protein. Aim: to investigate the expression patterns of class II histone deacetylases (HDACs) in epithelial ovarian cancer cell lines, with a focus on evaluating their potential roles in tumor biology through mRNA and protein profiling, and to compare these findings with normal ovarian epithelial cells and existing public datasets for validation. Results: HDAC1 was significantly upregulated in SKOV-3 compared to OAW42 (1.7- and 1.8-fold vs. 0.5- and 0.24-fold, normalized to GAPDH and HPRT, respectively; p=0.04). HDAC2 expression was slightly downregulated in both lines, with no significant changes. HDAC3 showed significant upregulation in SKOV-3 (3.79-fold; p=0.02) and lower expression in OAW42. HDAC5 and HDAC6 were markedly upregulated in SKOV-3 (3.97- and 4.9-fold respectively; p=0.02), with moderate expression in OAW42. HDAC7 showed moderate upregulation in SKOV-3 (1.86-fold; p=0.12) and was not significantly altered in OAW42. HDAC8 was expressed at low levels in both cell lines with no significant differences. HDAC5 showed strong cytoplasmic staining in SKOV-3 and moderate to strong expression in HOSEpiC cells, while expression was low in OAW42. In contrast, HDAC6 exhibited stronger staining in OAW42 and HOSEpiC cells compared to moderate expression in SKOV-3. These findings partially align with the Human Protein Atlas data, which reported low expression of both proteins in ovarian cancer tissue. Our findings demonstrate that HDAC1, HDAC3, HDAC5, and HDAC6 are upregulated at the mRNA and/or protein levels in SKOV-3 ovarian cancer cells, with distinct expression patterns compared to OAW42 and HOSEpiC cells. These differential expression profiles suggest a potential role for specific HDACs, particularly HDAC5 and HDAC6, in ovarian cancer biology. However, discrepancies with Human Protein Atlas data highlight the need for further validation in clinical samples.
Keywords. HDACs, EOC, ICC, QRT-PCR.

Introduction

Despite massive efforts in early detection and new therapeutic approaches aimed at reducing the high death rate of ovarian cancer cases, ovarian cancer remains the deadliest gynecologic malignancy [1-3]. It is considered the eighth most common cancer in women globally and a leading cause of death, particularly in postmenopausal women, due to malignancies in females [4,5,28,29]. In 2022, approximately 21,179 new cases were diagnosed with ovarian cancer were diagnosed in the US, and 13,273 women died from the disease [28,29]. While ovarian cancer accounts for a smaller percentage of all gynecological cancer cases (around 2.5%), its high mortality rate contributes to a disproportionate 5% of cancer deaths [2,28]. Over 50% of ovarian cancer cases are detected at an advanced stage, leading to a poor long-term prognosis, as clinical signs are often nonspecific [4,6,30,31]. The challenges in early ovarian cancer detection mean that a significant majority, estimated at 4 out of 5 ovarian carcinomas, are discovered at late stages after the cancer has spread beyond the abdominal cavity [7,30,32].

In particular, 80% to 90% of malignant ovarian tumors originate from the single layer of epithelial cells covering the ovaries [4,8-12,33,34]. Thus, epithelial ovarian cancer (EOC) comprises most malignant ovarian neoplasms [8,35] and is the eighth most frequently diagnosed cancer globally and the leading cause of death from gynecologic cancer in the United States [10,13,14,28,29]. More than 70% of EOC cases present with specific symptoms at advanced stages, yet are still identified only during these late stages due to the

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insufficient sensitivity of current specific markers and various diagnostic methods for early detection [15,30,31,35].

According to histone deacetylation, one of the transcriptional silencing mechanisms in ovarian cancer [16,36,44], there is a relationship between HDACs and several well-distinguished cellular oncogenes and tumor-suppressor genes that influence an abnormal recruitment of HDAC activity, which in turn causes changes in gene expression [17,18,37,38]. Based on HDACs' homology to yeast proteins, the 18 HDACs found in humans are classified into four main classes, as there is cumulative proof that not all of them play essential functions. These classes are: Class I (HDAC1, HDAC2, HDAC3, and HDAC8), which correlate to yeast RPD3 (Saccharomyces cerevisiae) and are expressed in most cell types; Class II, which includes HDAC4, HDAC5, HDAC7, and HDAC9 (homologous to yeast HDA1), and Class IIb, comprising HDAC6 and HDAC10, which contain two catalytic locations. The expression pattern of Class II HDACs is more limited, and they are thought to play roles in cellular differentiation and developmental processes. Class III HDACs (Sirtuins) have homology to yeast Sir2 and have an absolute requirement for NAD+ [45,46], and Class IV (HDAC11) has conserved residues in its catalytic center similar to Class I and Class II deacetylases [19-23,24,36,39,40]. Specifically, in the HDACII class, phosphorylation and interaction with connector proteins like 14-3-3 induce HDACII nuclear export, facilitated by shuttling Class II HDACs between the nucleus and cytoplasm. As a reaction to numerous signals, these specific procedures suggest that Class II HDACs might be involved in cellular differentiation and developmental processes, and their impairment could contribute to carcinogenesis [25-27,41,42,47].

Due to the limited expression criterion of Class II HDACs, even the variation of their expression level and their connection in clinical trials has not been fully elucidated [27,41,47,48]. Therefore, HDAC Class II has been chosen among the HDAC family to determine if they could be utilized as early diagnostic biomarkers for Epithelial Ovarian Cancer (EOC) [41,42,47]. In this study, the expression profiling levels of HDAC1, 2, 3, 5, 6, 7, and 8 were compared through RT-qPCR among human epithelial ovarian cancer cell lines, concerning the normal epithelial ovarian cell line. In addition, immunocytochemistry was utilized for detecting the protein of gene expression of HDAC5 and HDAC6 in those cell lines. This limitation was primarily due to budgetary constraints and the limited availability of specific antibodies for the remaining HDACs. The cost and of obtaining high-quality antibodies suitable for immunocytochemistry made it challenging to expand the analysis to all targeted HDACs. Nonetheless, the choice of HDAC5 and HDAC6 was based on their relevance and potential significance in EOC pathogenesis as indicated in previous literature [43,49,50]. This study aims to evaluate the differential expression patterns of class II histone deacetylases (HDACs)—specifically HDAC1, HDAC2, HDAC3, HDAC5, HDAC6, HDAC7, and HDAC8—at both the mRNA and protein levels in epithelial ovarian cancer cell lines (SKOV-3 and OAW42), and to compare them with normal ovarian epithelial cells (HOSEpiC). This investigation seeks to determine the potential roles of these HDACs in ovarian tumor biology and assess their value as diagnostic biomarkers by integrating experimental data with public genomic datasets for cross-validation.

Materials and Methods

Cell culture

The human ovarian cancer cell lines used in this study were purchased from Prof. Hiss's lab. SKOV-3 cells and OAW42 were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS), glucose (4.5 g/L), penicillin G (100 U/ml), streptomycin (2.5 g/mL), glutamine (2 mmol/L), and fungizone (2.5 g/mL). Cells were incubated at 37°C in a humidified atmosphere. These cells were grown up to 80% confluence, harvested by trypsinization, and resuspended in (Ca^{2+}/Mg^{2+})-free phosphate-buffered saline (PBS). In comparison to a control cell line, HOSEPiC is described by Grizzle (1988 #3): about 10-20 ml of complete Ovarian Epithelial Cell Medium (OEpiCM) Catalog #7311 was added to the T-75 flask, which was prepared by adding both of the supplement tubes to the basal medium via pipette. Later, the HOSEpiC cryovial was brought, and the thawed contents of the 1 ml cryovial were re-suspended and poured carefully into the equilibrated, poly-L-lysine-coated culture vessels. A seeding density is more than 5*10^5 cells/cm2; 1 ml/vial. In the incubator, the cells were left with 5% CO_2 and 37°C.

RNA extraction and real-time qPCR analysis

Total RNA was extracted from SKOV3, OAW42, and HOSEPiC cells using the RNeasy Mini Kit (Roach). Seven micrograms of total RNA from each Skov-3 and OAW42 sample and five micrograms from the HOSEPIC sample were utilized for reverse transcription using the iScript cDNA synthesis kit (Bio-Rad, Hercules, USA). Purity and the amount of RNA and cDNA were measured using a Nanodrop spectrophotometer and agarose gel electrophoresis. Real-time PCR was carried out on cDNA using KAPA SYBR® FAST qPCR Master Mix (2X) KAPABIOSYSTEMS with the Light Cycler 480 (Roche). All reactions were performed in a 20 µL volume. PCR was performed by an initial denaturation at 58°C for 5 min, followed by 35 cycles for 30 s at 95°C, 30 s at 60°C, and 30 s at 72°C. PCR using water instead of the template was used as a negative control. Specificity verified by melting curve analysis and agarose gel electrophoresis. The expression of the related HDACs was measured using a KAPA SYBR® FAST for ABI Prism® PCR Mix (2X) (Kapa Biosystems). The threshold cycle

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values of each sample were used in the post-PCR data analysis. GAPDH RNA is used as an internal control for mRNA-level normalization. Standard curves were made using serially diluted cDNA of one SKOV3, OAW42, and HOSEPiC. Throughout the NCBI website, the primer sequencing was designed for the studied HDACs, and using GAPDH and HPRT as internal control genes, after annealing temperature optimization by using PCR, the target genes with reference genes were investigated by electrophoresis on a 1.5% agarose gel and imaged by GEL RED.

Immunocytochemistry

OAW42, SKOV-3, and HOSEpiC cells were seeded onto sterile, autoclaved, positively charged glass slides (76 mm x 26 mm; 1.0 mm to 1.2 mm thick) and maintained in DMEM medium supplemented with 5% fetal bovine serum at 37°C in a humidified, 5% CO2 atmosphere for SKOV-3 and OAW42 and OPIMIC media for HOSEpiC. At 50% confluence, these cells had to be washed with 1X 1% PBS for three minutes, and then they were fixed by immersion in 4% paraformaldehyde (PFD) for 20 minutes at room temperature; consequently, the cells were washed 3 times with PBS for 5 minutes every single time. Later, the cells had to be stained with the Anti-GFRA1 antibody (1:500 dilution), and at the Histology Laboratory at Tygerberg Hospital, in a Leica Bond Autostainer (Leica), all staining, washing, and antibody incubations were performed. The mentioned antibodies used were obtained from Abcam and Thermo Fisher. Immunocytochemistry for the genes was done with an automated stainer (Benchmark XT, Ventana) following the protocols of the manufacturer. All analyses of immunocytochemical staining (Figures 9, 10) were carried out by investigators (Reggie Williams).

Statistical Analysis

The relative expression levels of each gene were normalized to the geometric mean of the two reference genes and scaled to the control group. A GraphPad and Pairwise Fixed Reallocation Randomization Test © software statistical analysis was performed for all samples. A value of $p < 0.05$ was statistically significant.

Results

RT_QPCR of the relative HDACs

The mRNA expression levels of HDAC1, 2, 3, 5, 6, 7, and 8 in the two ovarian cancer cell lines, adenocarcinoma (SKOV-3) and cystadenocarcinoma (OAW42), were investigated by normalization against the housekeeping genes (GAPDH and HPRT) in the normal human ovarian surface epithelial cell line (HOSEPiC) by qRT-PCR (Figure 2). The Ct values of the HKGs (GAPDH and HPRT) as measured in both the control cell line (HOSE) and the cancer cell lines (SKOV-3 and OAW42) were for the Ct value of HPRT1 expression, which was found to be relatively more stable for the 3 cell lines evaluated compared against GAPDH, which was a highly unstable expressed gene in the three different cell lines, as (Figure 1) shows, so for this reason the HPRT p-values were used to interpret the expression of genes was described in terms of the fold change up or down (Tables 1; Figures 3).

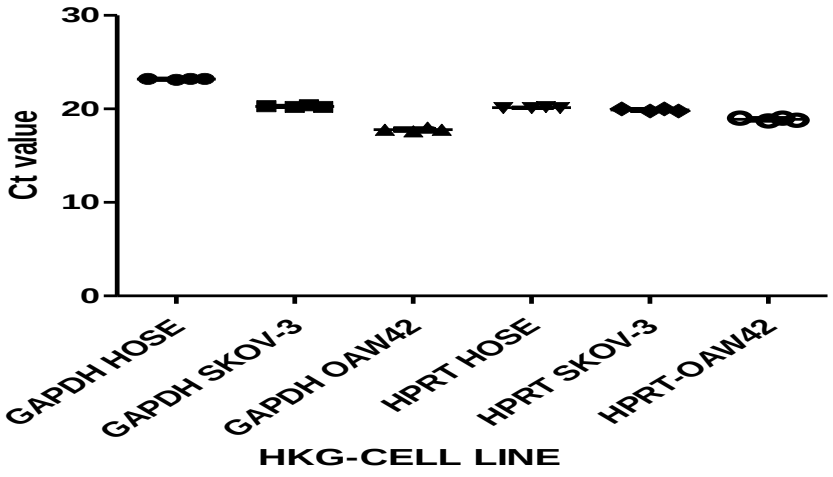

Figure 1: Q-PCR Ct values for different HKGs in different cell lines. Genes evaluated were GAPDH and HPRT.

HPRT fulfilled most criteria as a suitable housekeeping gene in that it was steadily expressed, and it displayed the least fluctuation, as shown in (Figure 2). Thus was the better HKG gene to use in the

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normalization of the target gene's expression. However, there were no significant differences in the relative expression of the candidates' genes when a comparison was made between the normalization against HPRT vs. GAPDH. Candidates' genes, when a comparison is made between the normalization against HPRT vs GAPDH.

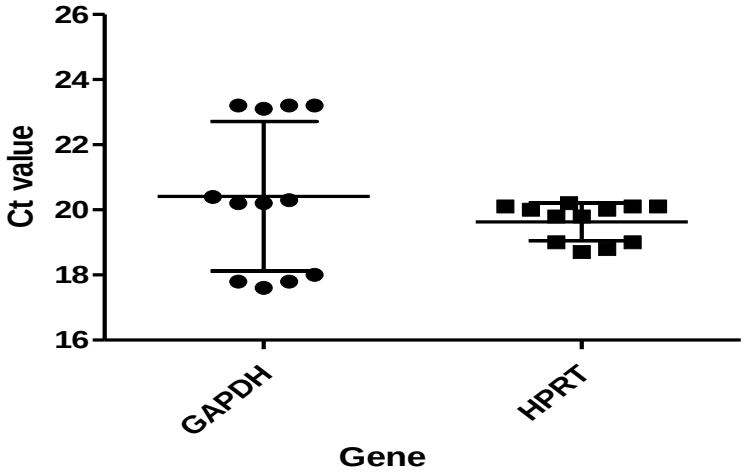

Figure 2: Combined Ct values for GAPDH and HPRT across all the cell lines.

An E value of two indicates 100% primer efficiency, and an R^2 value of 0.99 indicates a good correlation between Ct and sample concentration in HOSEpiC, SKOV-3, and OAW42. The gene expression intensities were quantified as fold change for each gene fragment, and the computed ratio Confidence intervals (CI) and p-values for the fold change were also calculated using GraphPad Prism and REST384© statistical analysis software. Variances were considered significant if $p \geq 0.05$. Real-time quantitative PCR (qRT-PCR) data demonstrated a 1.7-fold upregulated HDAC1 mRNA expression in the SKOV-3 (Figure 3) compared to the OAW42 cell line, which was 0.5-fold upregulated when normalized against GAPDH in both cell lines (Figure 5). Against HPRT, HDAC1 levels presented a 1.8-fold increase in SKOV-3 with p=0.04 (Figure 4), which were significantly upregulated when compared to low HDAC1 expression, i.e., 0.24-fold in the OAW42 cell line with p=0.08 (Figure 6).

Table 1: Analysis of candidate gene expression in SKOV-3 cells compared to HOSEpiC cells, normalized to GAPDH

Skov3 Relative to GAPDH	Gen	Absolute Regulation ratio	Log 2 ratio
HOSEpiC/SCOV-3	HDAC1	2.86	1.52-
HOSEpiC/SCOV-3	HDAC2	1.62	-0.7
HOSEpiC/SCOV-3	HDAC3	1.624	-3.55
HOSEpiC/SCOV-3	HDAC5	0.07	-3.73
HOSEpiC/SCOV-3	HDAC6	25.28	-4.66
HOSEpiC/SCOV-3	HDAC7	3.07	-1.62
HOSEpiC/SCOV-3	HDAC8	1.45	-0.54

The levels of HDAC2 mRNA in SKOV-3, normalized to GAPDH expression, were decreased 0.7-fold (Figure 3). Also, it represented a 1.04-fold decrease in OAW42 (Figure 5). Normalizing to HPRT, HDAC2 showed downregulated mRNA expression in SKOV-3, equivalent to a 0.94-fold change (p=0.07; Figure 4), matching OAW42, which expressed HDAC2 at a significantly lower level, i.e., a 1.79-fold change (p=0.13; Figure 6).

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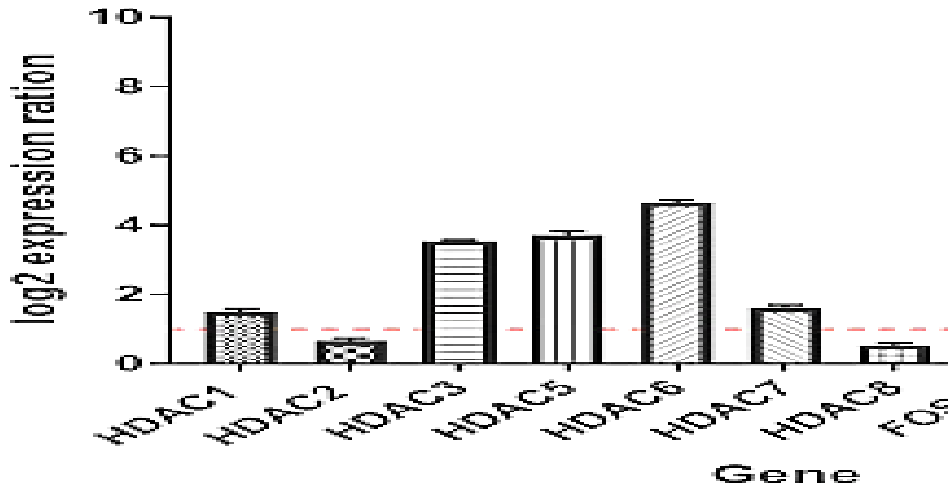

Figure 3: Relative expression ratio of target genes in SKOV-3 relative to the control cell line HOSE, normalized against GAPDH.
Red dotted lines indicate the cut-off for 2-fold expression difference.

Table 2: Comparison of differentially expressed fold change.
Analysis of candidates' gene expression in SKOV-3 compared to HOSEpiC, normalized to HPRT.

Skov3 Relative to HPRT	Gen	Absolute Regulation ratio	Log 2 ratio
HOSEpiC/SCOV-3	HDAC1	3.38	-1.76
HOSEpiC/SCOV-3	HDAC2	1.918	-0.94
HOSEpiC/SCOV-3	HDAC3	13.83	-3.79
HOSEpiC/SCOV-3	HDAC5	15.67	-3.97
HOSEpiC/SCOV-3	HDAC6	29.85	-4.9
HOSEpiC/SCOV-3	HDAC7	3.63	-1.86
HOSEpiC/SCOV-3	HDAC8	1.71	-0.78

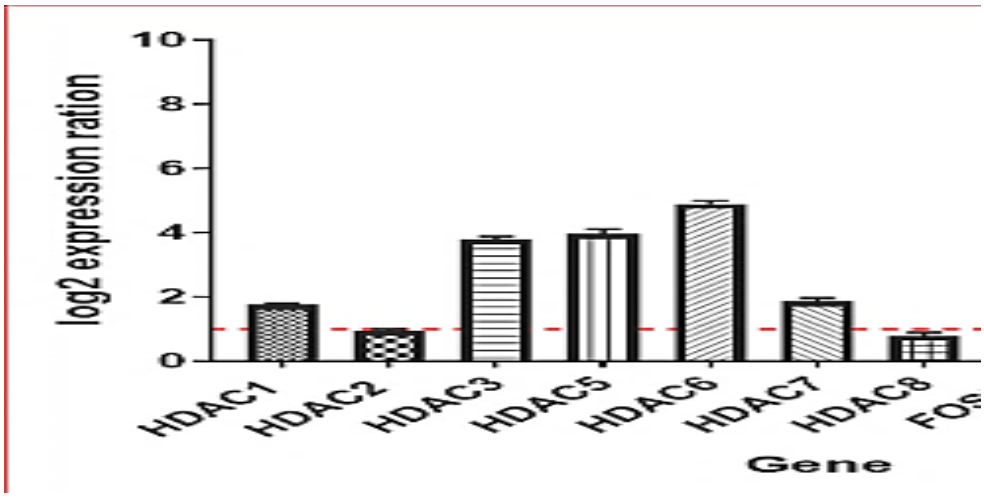

Figure 4: Relative expression ratio of target genes in SKOV-3 relative to the control cell line HOSE normalized against HPRT.
Red dotted lines indicate the cut-off for 2-fold expression difference.

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Table 3: Analysis of candidate gene expression in QAW42 cells compared to HOSEpiC cells, normalized to GAPDH

OAW42 Relative to GAPDH	Gen	Absolute Regulation ratio	Log 2 ratio
HOSEpiC/OAW42	HDAC1	1.42	-0.51
HOSEpiC/OAW42	HDAC2	0.48	1.04
HOSEpiC/OAW42	HDAC3	0.54	0.88
HOSEpiC/OAW42	HDAC5	0.07	-3.73
HOSEpiC/OAW42	HDAC6	3.94	-1.98
HOSEpiC/OAW42	HDAC7	3.94	-1.17
HOSEpiC/OAW42	HDAC8	1.40	-0.49

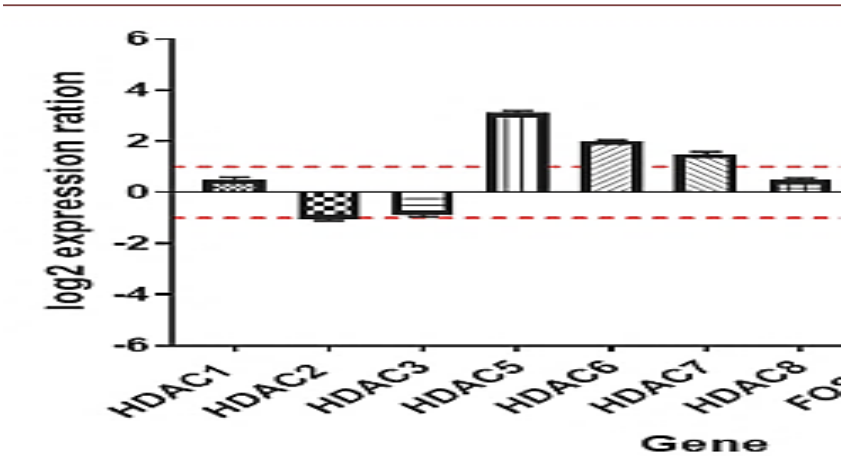

Figure 5: Relative expression ratio of target genes in OAW42 relative to the control cell line HOSE, normalized against GAPDH.

Red dotted lines indicate the cut-off for a 2-fold expression difference. Values below the zero line represent down-regulation of genes.

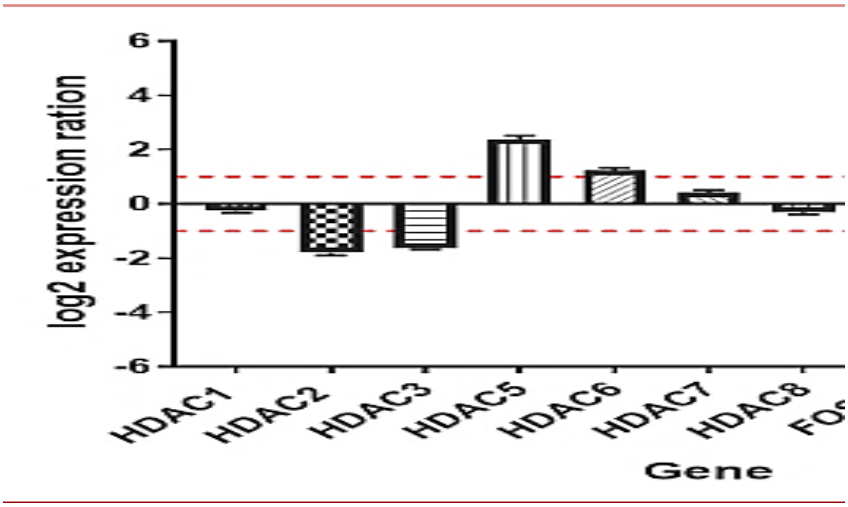

Figure 6: Relative expression ratio of target genes in OAW42 relative to the control cell line HOSE normalized against HPRT.

Red dotted lines indicate the cut-off for 2-fold expression difference. Values above zero represent up-regulation of genes. Values below the zero line represent down-regulation of genes.

HDAC3 mRNA was expressed at higher levels, i.e., a fold change of -3.55 in SKOV-3 to GAPDH normalization (Figure 3), but HDAC3 showed lower expression in OAW42, i.e., a 0.88-fold change (Figure 5). In contrast, HDAC3 mRNA expression was upregulated, as indicated by a 3.79-fold change in SKOV-3 (Figure 4) against HPRT, with p=0.02, and HDAC3 showed downregulated expression in QAW42 cells, as judged by a 1.63-fold change, which was not significant (p=0.08; Figure 6). HDAC5 mRNA was expressed at relatively lower levels, i.e., 3.7-fold in SKOV-3 cells relative to GAPDH, as shown in (Figure 3), while (Figure 5) depicts an increased HDAC5 mRNA expression level in OAW42, i.e., a 3.17-fold change. In (Figure 4), HDAC5 mRNA in SKOV-3 displayed an upregulated expression relative to HPRT, which was a 3.97-fold change with p=0.02. Also, HDAC5 mRNA expression was increased in OAW42, i.e., 2.37-fold change (p=0.05; Figure 6). As shown in

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(Figure 3), the relative density of HDAC6 mRNA was upregulated in SKOV-3, equivalent to a 4.66-fold change, and its expression was also high in OAW42, as indicated by a 1.98-fold change (Figure 5). Although HDAC6 expression was increased significantly relative to HPRT (4.9-fold change; p=0.02) in SKOV-3 cells (Figure 4), its expression in OAW42 was less (1.22-fold change; p=0.05), as shown in (Figure 6). Relative to GAPDH, HDAC7 mRNA was upregulated in SKOV-3 by a 1.6-fold change (Figure 3), similar to that observed for OAW42 cells (Figure 5). Against HPRT, HDAC7 mRNA expression showed a 1.86-fold increase in SKOV-3 cells (p=0.12; Figure 4), and it did not decrease significantly (0.4-fold change; p=0.31) in OAW42 cells (Figure 6). Analysis of HDAC8 mRNA expression revealed low levels in all samples tested. Notably, there was a 0.54-fold increase in SKOV-3 cells (Figure 3), which was relatively similar to the HDAC8 mRNA decrease in OAW42 cells as indicated by a 0.49-fold change (Figure 5). Thus, HDAC8 mRNA expression was not significantly downregulated in SKOV-3 cells (0.78-fold change; p=0.30; Figure 4). Likewise, in OAW42 cells, HDAC8 mRNA expression was decreased (-0.26-fold change; p=0.32; Figure 6).

Discussion

The Human Protein Atlas (HPA) is a comprehensive repository of protein expression profiles of normal and cancer tissues, as well as cell lines [33]. It also allows researchers to evaluate specific target genes as biomarkers for cancers due to the availability of whole transcriptomes created from the sequencing of RNA/transcripts (RNA-Seq) of tissue samples [34,35].

The 7 mentioned target genes were subjected to cross-cancer tissue-specific analysis in the HPA. HDAC8 was significantly upregulated selectively in ovarian cancer, as shown by HPA analysis. This does not agree with the qRT-PCR data generated in the current study since the qRT-PCR results suggest that the expression levels of the protein encoded by this gene are downregulated in SKOV-3 and OAW42 relative to control HOSEpiC cells. It is a known fact that gene expression and qRT-PCR data do not need to be the same, as there are many factors that contribute to this observation. Most studies have shown a 40% correlation between cellular concentrations of proteins and the abundance of their corresponding mRNA in both bacteria and eukaryotes [36]. Based on the qRT-PCR data, HDAC1, HDAC3, HDAC5, HDAC6, and HDAC7 were expressed highly in SKOV-3 relative to HOSEpiC cells, which correlates with HPA proteomics results in the Appendix, except for HDAC2 low gene expression level in SKOV-3 cells compared to its high expression level in HPA. Unlike in OAW42, expression of HDAC1 and HDAC7 genes was downregulated, which disagrees with the HPA data. In this respect, the qRT-PCR results show that HDAC2, HDAC3, HDAC5, and HDAC6 are expressed at higher levels in OAW42 relative to HOSEpiC, which is in agreement with HPA data.

(Figures 7 & 8). Give a graphical display of HDAC5 and HDAC6 genes and their expression in selected cancers, including ovarian carcinomas, for both databases. The HPA database compares the expression of genes in human cancer tissue types.

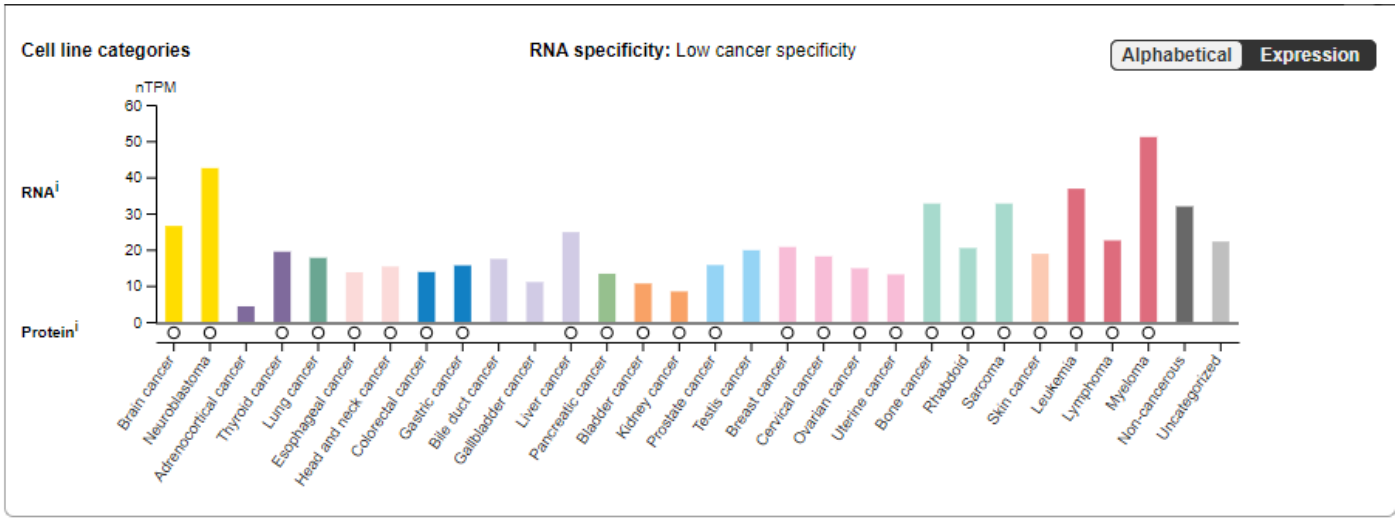

Figure 7: HDAC5 gene and its expression in selected cancers

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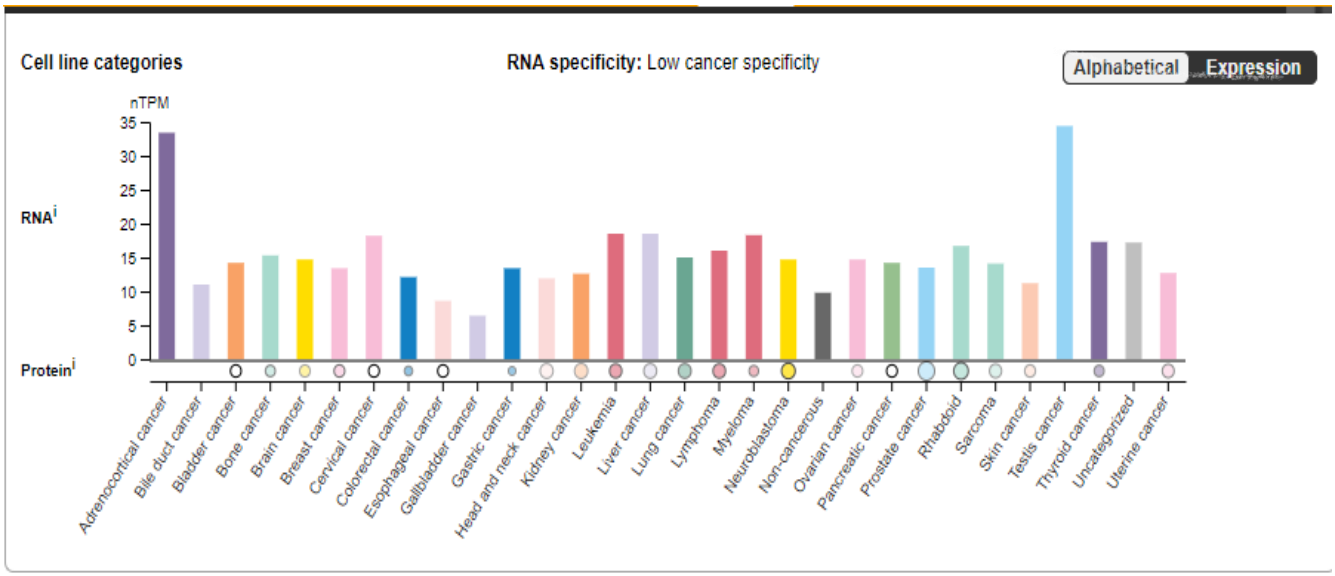

Figure 8: HDAC6 gene and its expression in selected cancers

Immunohistochemistry of HDAC5, and HDAC6

Cells were stained with haematoxylin and eosin (H&E). A, B, and C show HOSEpiC-, OAW42-, and SKOV-3-cell cytoplasm, respectively, and stained with H&E and the HDAC5 antibody. The dark purple dye means the nuclei, whereas the brown stain represents HDAC5. The black arrows point to the localization of HDAC5. Images were taken at 20X magnification (Figure 9). It shows that the expression levels of HDAC5 were high in the SKOV-3 cell cytoplasm and moderate to strong in the HOSEpiC cell cytoplasm. The expression of this gene was much lower in the OAW42 cell cytoplasm when compared to the level of intensity in the SKOV-3 and HOSEpiC cells. The staining intensity of HDAC6 proteins was stronger in OAW42 and HOSEpiC cell cytoplasm when compared to the expression in the cytoplasm of SKOV-3, which showed moderate intensity (Figure 10).

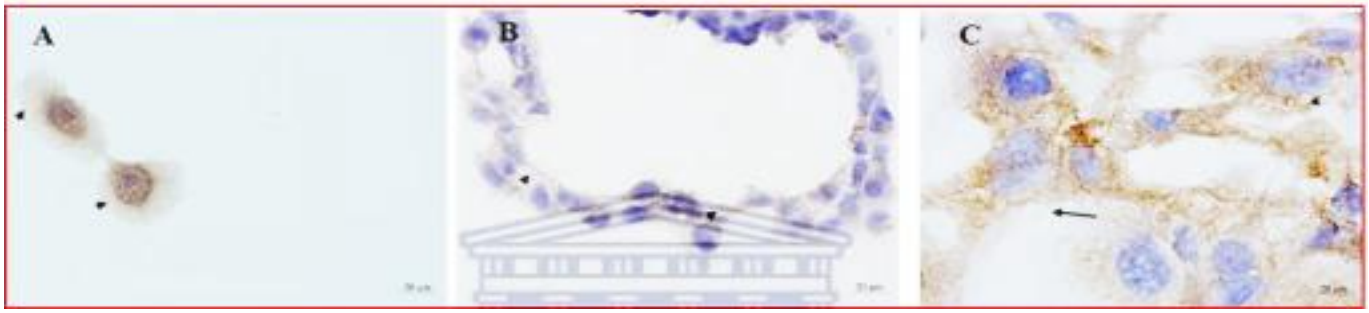

Figure 9: ICC analysis of HDAC5 expression in HOSEpiC, OAW42 and SKOV-3 cells

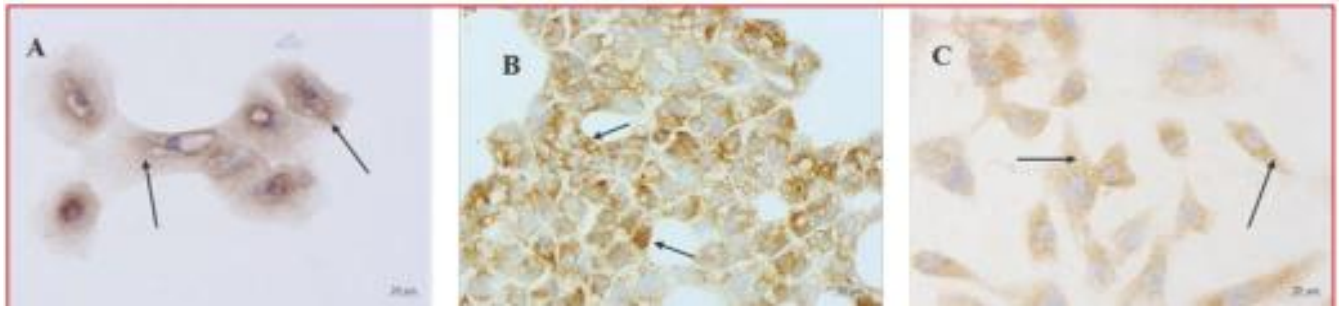

Figure 10: ICC analysis of HDAC6 expression in HOSEpiC, OAW42 and SKOV-3 cells

Also, the Human Protein Atlas analysis found that the mentioned genes were expressed at low levels in ovarian cancer tissue, which agrees with some of our ICC results. HDAC5 showed high-intensity staining in SKOV-3 cells, which is in disagreement with the HPA (Figure 9). Moreover, HDAC6 gave high cytoplasmic staining in OAW42 and moderate protein expression in SKOV-3, which does not corroborate with the HPA database investigation (Figures 9 and 10).

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Conclusion

In conclusion, while the HPA provides an invaluable resource for cancer biomarker research, our comparative analysis demonstrates that careful validation in specific experimental systems remains essential. The partial correlations and notable discrepancies between our findings and HPA data underscore the complexity of HDAC regulation in ovarian cancer and suggest that therapeutic targeting of these epigenetic modifiers will require careful consideration of cancer subtype-specific expression patterns. These results contribute to the growing understanding of HDAC biology in ovarian cancer and highlight the need for integrated multi-platform approaches in biomarker discovery and validation. The use of marker panels, including RT-QPCR and ICC tests, increased the sensitivity to 60% while maintaining 100% specificity. Hence, although further studies are required, our data recommend that a limited number of markers in combination might detect most ovarian cancers.

Conflict of interest. Nil

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