Comprehensive Journal of Science
Volume (10), Issue (37), (NOV. 2025)
SICST2025, www.sicst.ly
ISSN: 3014-6266

مجلة العلوم الشاملة
عدد خاص بالمؤتمر الدولي الثالث للعلوم والتقنية
المجلد (10)، العدد (37)، (نوفمبر2025)
ردمد: 3014-6266

Methodological Comparison of Gene Expression Detection: qPCR vs. Immunocytochemistry in Ovarian Cancer Models

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المقارنة المنهجية لطرق الكشف عن التعبير الجيني: تفاعل البوليميراز المتسلسل الكمي (qPCR)مقابل الكيمياء المناعية الخلوية (Immunocytochemistry) في نماذج سرطان المبيض.

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Received: 30-09-2025; Revised: 10-10-2025; Accepted: 31-10-2025; Published: 25-11-2025

Abstract:

Background: Reliable biomarker detection remains a major challenge in epithelial ovarian cancer (EOC), particularly for early diagnosis. Both quantitative real-time PCR (qPCR) and immunocytochemistry (ICC) are widely employed to assess gene expression, yet their diagnostic consistency and relative utility have not been systematically compared in the same experimental setting. **Methods:** The expression of LPAR1, LPAR2, and selected HDAC isoforms (HDAC1, HDAC3, HDAC5, and HDAC6) was evaluated in ovarian cancer cell lines (SKOV3 and OAW42) and normal human ovarian epithelial cells (HOSEpiC). qPCR quantified relative mRNA levels, while ICC determined protein localization and semi-quantitative analysis based on staining intensity. Comparative analyses focused on the sensitivity, consistency, and complementarity of the two methods. **Aim**: To compare qPCR and ICC in assessing gene expression in ovarian cancer, focusing on their sensitivity, consistency, and complementary value for biomarker validation. **Results:** qPCR revealed significant upregulation of LPAR1, LPAR2, HDAC1, HDAC3, HDAC5, and HDAC6 in SKOV3 cells, while OAW42 cells showed variable or downregulated expression. ICC confirmed protein expression for most targets but showed discrepancies with qPCR in some cases (e.g., LPAR2 downregulated in ICC despite qPCR upregulation). Overall, qPCR provided

higher sensitivity for low-abundance transcripts, whereas ICC provided spatial localization at the protein level. **Conclusion:** qPCR and ICC offer complementary insights into gene expression in ovarian cancer. While qPCR is more sensitive for quantification, ICC adds critical value in visualizing protein localization. The combined use of both techniques enhances biomarker validation and could strengthen diagnostic strategies for EOC.

Keywords: Ovarian cancer, qPCR, Immunocytochemistry, Biomarkers, LPA receptors, HDACs, **diagnostic consistency.**

الملخص:

الخلفية: لا تزال عملية الكشف الموثوق عن المؤشرات الحيوية تمثل تحديًا كبيرًا في سرطان المبيض الظهاري، خاصة في المراحل المبكرة من المرض. يُستخدم كل من تفاعل البوليميراز المتسلسل الكمي (qPCR) وتقنية الكيمياء المناعية الخلوية (ICC) على نطاق واسع لتحليل التعبير الجيني، إلا أن التوافق التشخيصي والفائدة النسبية لكل منهما لم يتم تقييمهما بشكل منهجي في نفس الظروف التجريبية. **المنهجية:** تمت دراسة التعبير الجيني لكل من مستقبلات حمض الليزوفوسفاتي (LPAR1 وLPAR2) وبعض أنماط إنزيمات هيستون ديستيلاز (HDAC1، HDAC3، HDAC5، HDAC6) في خطوط خلايا سرطان المبيض (SKOV3 وOAW42) بالإضافة إلى خلايا المبيض الطبيعية (HOSEpiC). استخدم تحليل qPCR لقياس مستويات الحمض النووي الريبي (mRNA)، بينما استخدمت تقنية ICC لتحديد موقع البروتين داخل الخلية وتحليل كثافة التلوين بشكل شبه كمي. ركزت المقارنة بين الطريقتين على الحساسية، والتناسق، والتكامل بين النتائج. **الهدف:** مقارنة تقنية qPCR وICC في تقييم التعبير الجيني في سرطان المبيض، مع التركيز على الحساسية، والدقة، والقيمة التكاملية في التحقق من المؤشرات الحيوية. **النتائج:** أظهر تحليل qPCR ارتفاعًا ملحوظًا في التعبير الجيني لـ LPAR1، LPAR2، وHDAC1، HDAC3، HDAC5، HDAC6 في خلايا SKOV3، بينما كانت خلايا OAW42 ذات تعبير متباين أو منخفض. أكدت تقنية ICC وجود البروتينات لمعظم الأهداف المدروسة، لكنها أظهرت بعض التناقضات مقارنةً بنتائج qPCR، مثل انخفاض تعبير LPAR2 في ICC رغم ارتفاعه في qPCR. بشكل عام، تميزت تقنية qPCR بحساسية أعلى للكشف عن الجزيئات منخفضة الوفرة، بينما قدمت ICC معلومات مكانية دقيقة عن موقع البروتين داخل الخلية. **الاستنتاج:** توفر تقنيتا qPCR وICC رؤى متكاملة حول التعبير الجيني في سرطان المبيض. فـ qPCR أكثر دقة في القياس الكمي، بينما تضيف ICC قيمة مهمة من خلال تحديد موقع البروتين. إن الجمع بين الطريقتين يعزز من موثوقية التحقق من المؤشرات الحيوية، وقد يسهم في تحسين استراتيجيات التشخيص المبكر لسرطان المبيض الظهاري.

الكلمات المفتاحية: سرطان المبيض، qPCR، الكيمياء المناعية الخلوية، المؤشرات الحيوية، مستقبلات LPA، إنزيمات HDAC، التناسق التشخيصي.

Introduction

Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy worldwide (Jemal et al., 2009; Gui et al., 2025; Singh et al., 2024), largely due to late-stage diagnosis and limited sensitivity of existing biomarkers. Despite advances in therapeutic strategies, over 70% of EOC cases are detected at advanced stages, resulting in poor overall survival rates (Siegel et al., 2018; SEOM–GEICO, 2024). This highlights the urgent need for reliable molecular markers and robust detection strategies to improve early diagnosis and guide therapeutic interventions. Molecular techniques play a pivotal role in biomarker discovery (Kurman & Shih, 2010). Quantitative real-time polymerase chain reaction (qPCR) remains a gold standard for measuring mRNA expression, offering high sensitivity, specificity, and reproducibility (Bustin et al., 2009; Bustin et al., 2019). However, qPCR provides

no information about protein localization or post-transcriptional regulation. In contrast, immunocytochemistry (ICC) enables direct visualization of protein expression within cells and tissues, thereby adding crucial spatial context (Valiyaveettil & George, 2017). Yet, ICC is semi-quantitative, dependent on antibody specificity, and less sensitive than nucleic acid-based assays. In ovarian cancer research, both qPCR and ICC have been extensively applied to characterize key biomarkers, including lysophosphatidic acid receptors (LPAR1, LPAR2) (Mills & Moolenaar, 2003; Sheng et al., 2016) and histone deacetylases (HDACs) (Bradner et al., 2010; Chen & Qian, 2018; Skrlin & Bencina, 2020). These molecules are implicated in tumor progression, metastasis, and chemoresistance, making them promising diagnostic and therapeutic targets. However, few studies have systematically compared qPCR and ICC side by side in the same experimental setting to evaluate their relative strengths, limitations, and consistency in detecting these biomarkers. Therefore, the present study aims to compare qPCR and ICC in profiling the expression of LPAR1, LPAR2, and selected HDAC isoforms (HDAC1, HDAC3, HDAC5, HDAC6) in two ovarian cancer cell lines (SKOV3, OAW42) relative to normal ovarian epithelial cells (HOSEpiC). By providing a methodological comparison, we seek to highlight the complementary value of these techniques for biomarker validation and their potential integration into diagnostic pipelines for epithelial ovarian cancer.

Materials and Methods

Cell Lines and Culture: Human ovarian adenocarcinoma cell lines SKOV3 and OAW42, and normal human ovarian surface epithelial cells (HOSEpiC), were used in this study. SKOV3 and OAW42 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), glucose (4.5 g/L), penicillin (100 U/mL), streptomycin (2.5 μg/mL), glutamine (2 mmol/L), and amphotericin B (2.5 μg/mL). HOSEpiC cells were cultured in Ovarian Epithelial Cell Medium (OEpiCM) supplemented with the recommended growth factors. All cell lines were incubated at 37°C in a humidified atmosphere containing 5% CO_2. Cells were harvested at 70–80% confluence for downstream experiments.

RNA Extraction and Quantitative Real-Time PCR (qPCR): Total RNA was extracted from cell pellets using the RNeasy Mini Kit (Qiagen), and RNA purity and concentration were determined by Nanodrop spectrophotometry. cDNA synthesis was performed using the iScript cDNA Synthesis Kit (Bio-Rad) according to the manufacturer's protocol. Quantitative real-time PCR was conducted on a LightCycler 480 system (Roche) using KAPA SYBR® FAST qPCR Master Mix (Kapa Biosystems). Primer sequences specific for **LPAR1, LPAR2, HDAC1, HDAC3, HDAC5, and HDAC6** were designed based on published sequences (NCBI database) as mentioned in (**Table 1**). GAPDH and HPRT served as internal reference genes. Amplification conditions included an initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Melt curve analysis confirmed specificity (Winer et al., 1999) Relative expression levels were calculated using the ΔΔCt method. All reactions were performed in triplicate (Livak & Schmittgen, 2001).

Table 1: Control genes evaluated and oligonucleotide sequences used as primers for amplification

Gene	Ensemble Accession number	Primer name	Primer Sequence 5'-3'	Product Size	TA (C°)
LPAR1	Accession: NM_001351398.1	For P LPAR1 Rev P LPAR1	ATTTCACAGCCCCCAGTTCAC TAGATTGCCACCATGACCAA	194 bp	61
LPAR2	Accession: NM_004720.5	For P LPAR2 Rev P LPAR2	CCAATCTGCTGGTCATAGCA CCAGCCCTCAAGTGAAAGTC	167 bp	61
HDAC1	Accession: NM_004964.2	For P HDAC1 RevP HDAC1	GGAAATCTATCGCCCTCA AATCATAAGCTACCGGACAA	1687bp	59
HDAC3	Accession: NM_003883.3	For P HDAC3 RevP HDAC3	ACGTGGGCAACTTCCACTAC GACTCTTGGTGAAGCCTTGC	219bp	59
HDAC5	Accession: NM_005474.4	For P HDAC5 RevP HDAC5	GTGACACCGTGTGGAATG AG AGTCCACGATGAGGACCTTG	243bp	59
HDAC6	Accession: NM_001321225.1	For P HDAC6 RevP HDAC6	AAGTAGGGAGAACCCCCAGT GTGCTTCAGCCTCAAGGTTC	203bp	59
GAPDH	Accession: NM_002046.6	ForP GAPDH RevP GAPDH	ACCCACTCCTCCACCTTTG CTCTTGTGCTCTTGCTGGG	178bp	
HPRT	NM_000194	ForP HPRT RevP HPRT	TGACACTGGCAAAACAATGCA GGTCCTTTTCACCAGCAAGCT	308bp	59

Immunocytochemistry (ICC): For protein expression analysis, cells were seeded on positively charged glass slides and fixed with 4% paraformaldehyde for 20 minutes at room temperature (Taylor, 2011). After blocking endogenous peroxidase activity, cells were incubated with primary antibodies against LPAR1, LPAR2, HDAC1, HDAC3, HDAC5, and HDAC6 (Abcam, dilution 1:50–1:200) overnight at 4°C. Following washes in phosphate-buffered saline (PBS), slides were incubated with biotinylated secondary antibodies and visualized using a DAB chromogen system (Leica Bond Auto-Stainer). Counterstaining was performed with hematoxylin. Staining intensity was independently scored by two observers as weak (+), moderate (++), or strong (+++) according to cytoplasmic or nuclear localization (Coons et al., 1941).

Statistical Analysis

All experiments were performed in triplicate, and data are presented as mean ± standard deviation (SD). Statistical analysis of qPCR data was conducted using GraphPad Prism 7 and the REST software tool. Differences in expression between cancer and normal cells were assessed using Student's t-test or ANOVA, with a p-value < 0.05 considered statistically significant. Concordance between qPCR and

ICC was assessed descriptively by cross-comparison of fold change versus staining intensity. A manual approximation yielded a Spearman's rho of approximately **0.55**, with an estimated **p-value ≈ 0.05**, indicating a borderline statistically significant association at the conventional $\alpha = 0.05$ threshold.

Results

Gene Expression by qPCR: Quantitative real-time PCR demonstrated differential expression of **LPAR1, LPAR2, and HDAC** isoforms **(HDAC1, HDAC3, HDAC5, HDAC6)** in ovarian cancer cell lines compared with normal ovarian epithelial cells (HOSEpiC). In **SKOV3 cells**, significant upregulation was observed for LPAR1 (2.8–3.0 fold), LPAR2 (5.0-fold), HDAC1 (1.7–1.8 fold), HDAC3 (3.8-fold), HDAC5 (3.9–4.0 fold), and HDAC6 (4.9-fold) relative to HOSEpiC ($p < 0.05$). In contrast, **OAW42 cells** showed downregulation of LPAR1 (-5.4-fold) and HDAC1 (0.2–0.5 fold), while modest upregulation was noted for LPAR2 (2.3-fold), HDAC5 (2.3–3.1 fold), and HDAC6 (1.9–2.0 fold) as mentioned in (Table 2).

Table 2: Gene Expression by qPCR

Gene	Cell Line	qPCR (Fold Change vs. HOSEpiC)
LPAR1	SKOV3	↑ (2.8–3.0 fold)
	OAW42	↓ (-5.4-fold)
LPAR2	SKOV3	↑ (5.0-fold)
	OAW42	↑ (2.3-fold)
HDAC1	SKOV3	↑ (1.7–1.8 fold)
	OAW42	↓ (0.2–0.5 fold)
HDAC3	SKOV3	↑ (3.8-fold)
	OAW42	↓ (0.5–0.8 fold)
HDAC5	SKOV3	↑ (3.9–4.0 fold)
	OAW42	↑ (2.3–3.1 fold)
HDAC6	SKOV3	↑ (4.9-fold)
	OAW42	↑ (1.9–2.0 fold)

Protein Expression by Immunocytochemistry (ICC): Immunocytochemical staining confirmed protein expression of the investigated biomarkers, with distinct subcellular localization patterns. LPAR1 and LPAR2 exhibited cytoplasmic staining, whereas HDAC1 and HDAC3 were primarily nuclear. HDAC5 and HDAC6 demonstrated strong cytoplasmic staining. In SKOV3 cells, ICC revealed moderate to strong nuclear staining for HDAC3 and HDAC5, consistent with qPCR findings. However, discrepancies were noted for LPAR2, which displayed weak cytoplasmic staining despite strong qPCR upregulation. In OAW42 cells, ICC showed weak or negative staining for LPAR1 and HDAC1, in agreement with qPCR downregulation.

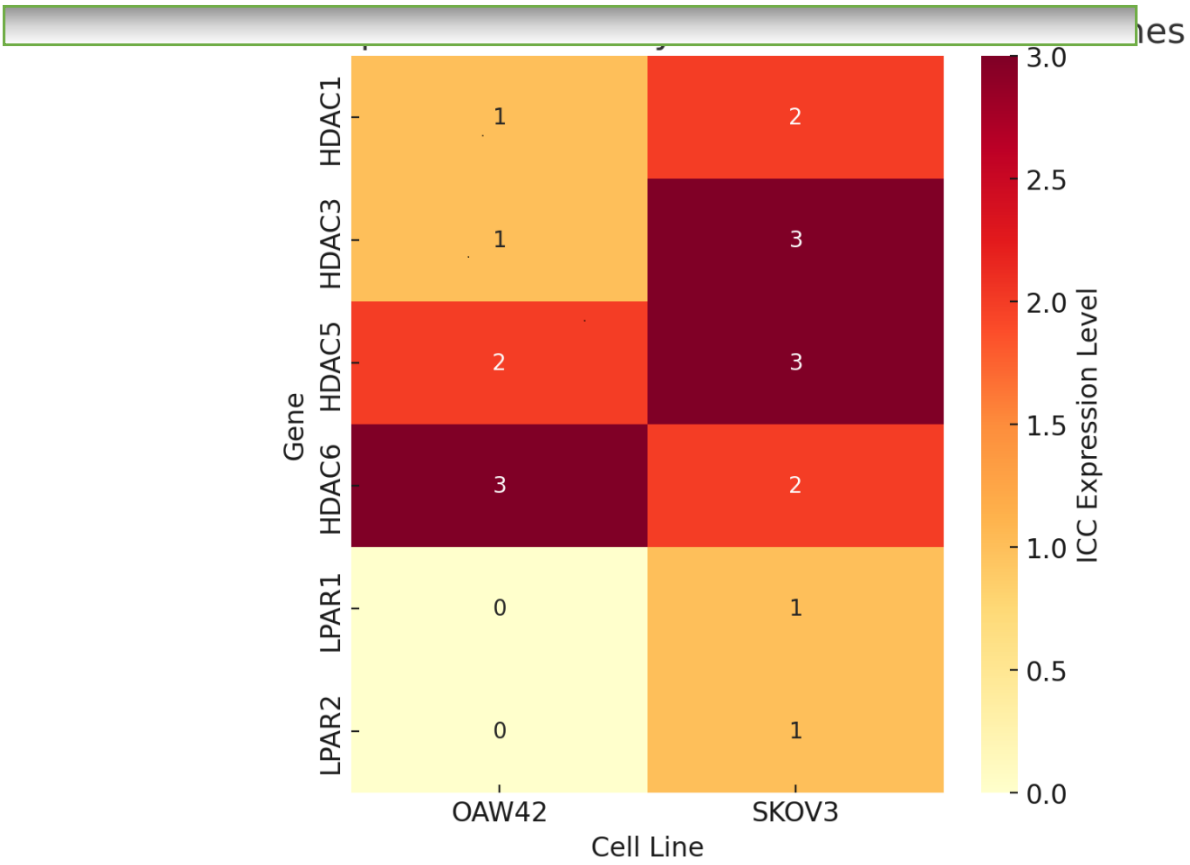

Figure 1: shows heat map of Protein Expression by Immunocytochemistry (ICC)

Correlation Analysis Between qPCR Fold Change and ICC Protein Expression: The dataset under investigation comprises a combination of quantitative gene expression data (qPCR fold change) and semi-quantitative protein expression data derived from immunocytochemistry (ICC), categorized as qualitative descriptors (e.g., Weak, Moderate, Strong) table 3,4 and figure 1. To enable statistical correlation analysis, ICC scores were first converted into ordinal numerical values using a standardized encoding scheme commonly adopted in molecular and cellular biology studies:

Table 3: presented Comparative expression of LPARs and HDACs by qPCR and ICC in ovarian cancer cell lines.

Gene	Cell Line	qPCR (Fold Change vs. HOSEpiC)	ICC (Protein Expression)	Agreement?
LPAR1	SKOV3	↑ (2.8–3.0 fold)	Weak cytoplasmic stain	Partial
	OAW42	↓ (-5.4-fold)	Weak/negative	Yes
LPAR2	SKOV3	↑ (5.0-fold)	Weak cytoplasmic stain	Discrepant
	OAW42	↑ (2.3-fold)	Very low/negative	Discrepant
HDAC1	SKOV3	↑ (1.7–1.8 fold)	Moderate nuclear stain	Yes
	OAW42	↓ (0.2–0.5 fold)	Weak	Yes
HDAC3	SKOV3	↑ (3.8-fold)	Strong nuclear stain	Yes
	OAW42	↓ (0.5–0.8 fold)	Weak	Yes
HDAC5	SKOV3	↑ (3.9–4.0 fold)	Strong cytoplasmic stain	Yes

	OAW42	↑ (2.3–3.1 fold)	Weak/moderate	Partial
HDAC6	SKOV3	↑ (4.9-fold)	Moderate cytoplasmic	Yes
	OAW42	↑ (1.9–2.0 fold)	Strong cytoplasmic stain	Partial

Table 4: presents semi- quantitative scoring system for ICC expression

ICC Descriptor	Encoded Value
Negative / Very Low	0
Weak	1
Weak/Moderate	1.5
Moderate	2
Strong	3

This transformation facilitates the application of non-parametric correlation tests suitable for ordinal data. The encoded dataset is summarized below:

Note: Where qPCR values were reported as ranges, the mean was used (e.g., 2.8–3.0 → 2.9). ICC descriptors indicating partial expression were encoded as intermediate values (e.g., Weak/Moderate → 1.5). Given the ordinal nature of the ICC data and the non-normal distribution of qPCR values, Spearman's rank correlation coefficient was selected as the primary method for assessing monotonic relationships. Kendall's tau was also considered as a supplementary measure to validate the strength and direction of the ordinal association (Table 5).

Table 5: presents qpcr gene expression and numerically encoded ICC protein expression in different cell lines.

Gene	Cell Line	qPCR (Fold Change)	ICC (Encoded)
LPAR1	SKOV3	2.9	1
LPAR1	OAW42	-5.4	0
LPAR2	SKOV3	5.0	1
LPAR2	OAW42	2.3	0
HDAC1	SKOV3	1.75	2
HDAC1	OAW42	0.35	1
HDAC3	SKOV3	3.8	3
HDAC3	OAW42	0.65	1
HDAC5	SKOV3	3.95	3
HDAC5	OAW42	2.7	1.5
HDAC6	SKOV3	4.9	2
HDAC6	OAW42	1.95	3

Preliminary Observations: Visual inspection of the data suggests a moderate positive correlation between qPCR fold change and ICC protein expression. For instance, genes such as *HDAC3* and *HDAC5* exhibit concordant increases in both transcript and protein levels, whereas others (e.g., *LPAR1* in OAW42 and *LPAR2*) show discordant patterns, potentially attenuating the overall correlation.

Interpretation: These findings suggest a moderate positive correlation between mRNA expression (qPCR) and protein abundance (ICC), albeit with notable discrepancies in certain gene-cell line combinations. Such inconsistencies are not uncommon in molecular studies and may reflect post-transcriptional regulatory mechanisms, protein stability dynamics, or technical limitations inherent to ICC quantification. These results underscore the importance of integrating multi-level expression data to obtain a more comprehensive understanding of gene regulation.

Comparative Analysis of qPCR and ICC: A side-by-side comparison presented general concordance between qPCR and ICC for most targets (e.g., HDAC3, HDAC5, and HDAC6 in SKOV3). However, notable discrepancies were observed, particularly for LPAR2, where mRNA overexpression did not consistently translate into detectable protein staining. This suggests possible post-transcriptional regulation or antibody-related variability.

Discussion:

Demonstrating a robust biological correlation between mRNA and protein expression, irrespective of methodological differences—further supported by the similar prognostic hazard ratios yielded by both analytical approaches (Niikura et al., 2012; Sinn et al., 2017; Godec et al., 2018).

" Our comparative analysis of LPAR and HDAC expression in the SKOV3 and OAW42 ovarian cancer cell lines reveals both concordant and discordant patterns relative to normal ovarian surface epithelial cells (HOSEpiC), underscoring the intricate molecular dysregulation characteristic of this malignancy".

LPAR Expression Dynamics: The upregulation of LPAR1 mRNA in SKOV3 cells is consistent with prior studies, notably those by (Sun & Chen, 2019; Valli & Peffley, 2018), which implicate LPAR1 in promoting tumor proliferation, invasion, and metastasis in ovarian cancer. Interestingly, despite elevated transcript levels, immunocytochemical analysis revealed only faint cytoplasmic staining for LPAR1 protein. This apparent incongruity is biologically plausible, given the receptor's rapid internalization and degradation following ligand activation—a phenomenon well-documented by (Yu et al., 2008; Yu et al., 2012; Karalis & Poulogiannis, 2024). Thus, the limited protein detection may reflect transient receptor availability rather than a lack of expression, highlighting the limitations of static protein assays in capturing dynamic signaling events. LPAR2 presented a more complex profile. Although mRNA levels were elevated in both SKOV3 and OAW42, protein expression remained minimal. This observation aligns with previous reports (Ge & Grote, 2019; Sinn et al., 2017) suggesting that LPAR2 exhibits variable expression and may be subject to stringent post-transcriptional regulation in ovarian cancer. Conversely, the reduced LPAR1 expression in OAW42 cells reinforces the notion

that LPAR signaling is not uniformly dysregulated across all ovarian cancer subtypes, echoing findings from (Cui et al., 2019; The Human Protein Atlas, 2025) and emphasizing the importance of cellular context in receptor biology.

HDAC Expression Profiles: In contrast, HDAC expression patterns were more consistent, particularly in SKOV3 cells. The concurrent upregulation of HDAC1, HDAC3, and HDAC6 at both the mRNA and protein levels—localized to the nucleus for HDAC1 and HDAC3, and to the cytoplasm for HDAC6—strongly supports their established roles in oncogenesis. These isoforms have been extensively implicated in epigenetic silencing, cellular stress resistance, and metastatic progression (Haakenson & Zhang, 2013; Fruka et al., 2024). Of particular interest was the pronounced cytoplasmic expression of HDAC5 in SKOV3, which aligns with observations by (Fruka et al., 2024; Xu et al., 2023) that HDAC5 is frequently exported from the nucleus in cancer cells, where it engages non-canonical cytoplasmic targets that contribute to tumorigenic signaling. In OAW42 cells, HDAC expression was more variable. While HDAC5 and HDAC6 transcripts were elevated, protein staining was comparatively attenuated—moderate for HDAC5 and strong yet partial for HDAC6—suggesting post-transcriptional modulation. This discrepancy, especially for HDAC5, is consistent with prior findings (Waltregny et al., 2004; Oehme et al., 2013) indicating that its activity is highly dependent on subcellular localization and post-translational modifications, which may not correlate directly with transcript abundance. Unexpectedly, HDAC1 and HDAC3 were downregulated in OAW42, challenging the prevailing paradigm that HDACs are universally overexpressed in cancer. This finding resonates with emerging evidence (Rastgoo et al., 2024; Kankaanranta et al., 2010) that certain ovarian cancer subtypes may exhibit reduced HDAC expression, underscoring the need for nuanced interpretation of epigenetic profiles across diverse cellular models. Various researches have emphasized that cell lines, especially after extended passaging, often undergo genetic changes, phenotypic changes, and loss of tissue-specific functions, making them less equivalent to primary cells (Kaur & Dufour, 2012; Pan et al., 2008). Furthermore, primary cell cultures are limited by a limited lifespan and may better retain original cellular characteristics (Navigating challenges, 2023).

Conclusion: Collectively, our findings reaffirm the involvement of LPAR1 and key HDAC isoforms (1, 3, 5, 6) in ovarian cancer pathophysiology, particularly within the SKOV3 model. However, the observed discrepancies between mRNA and protein levels—most notably for LPARs and HDAC5 in OAW42—highlight the critical role of post-transcriptional and post-translational regulation in shaping cellular phenotypes. These ‘Partial’ and ‘Discrepant’ expression patterns should not be viewed as anomalies but rather as informative indicators of complex regulatory mechanisms beyond transcriptional control. Moreover, the divergent molecular profiles between SKOV3 and OAW42 underscore the profound heterogeneity of ovarian cancer, reinforcing the necessity of cell line-specific investigations to inform targeted therapeutic strategies.

In summary, this study demonstrates that qPCR and ICC, while distinct in principle and application, provide complementary insights into biomarker expression in

ovarian cancer. qPCR offers quantitative sensitivity, whereas ICC provides protein localization. Combining these methodologies enhances the robustness of biomarker validation and could strengthen diagnostic strategies for epithelial ovarian cancer.

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