

Tumor Microenvironment and Cellular Adaptation in Ovarian Cancer Models Using Bio-Rad ddSEQ™ Single-Cell 3' RNA-Seq Technology

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Introduction

- High-grade serous carcinoma (HGSC) is one of the deadliest gynecological malignancies, exhibiting an 80% recurrence rate and high chemoresistance.¹
- Therapeutic responses to treatment remain limited, driven in part by the enrichment of persister cells. These subpopulation exhibit enhanced stem-like properties, increased proliferative capacity, and heightened metastatic potential.²
- In this study, we established patient-derived model of persister cells through repeated xenograft passages and patient-derived cultured cell lines. This strategy selectively enriches for highly aggressive cancer cells and recapitulates key molecular and phenotypic features of recurrent HCSC.
- We performed transcriptional profiling across these ovarian cancer model systems using single-cell RNA sequencing to resolve gene expression heterogeneity at single-cell resolution and capture distinct cellular states associated with tumor aggressiveness and recurrence.
- Selected transcriptional signatures were orthogonally validated using ddPCR system gene expression analysis, enabling highly sensitive and absolute quantification of key genes of interest. Together, these complementary approaches allowed for robust characterization of conserved and model-specific gene expression programs, providing insight into the translational relevance of each model system and informing their appropriate application in preclinical research.

Experimental Design

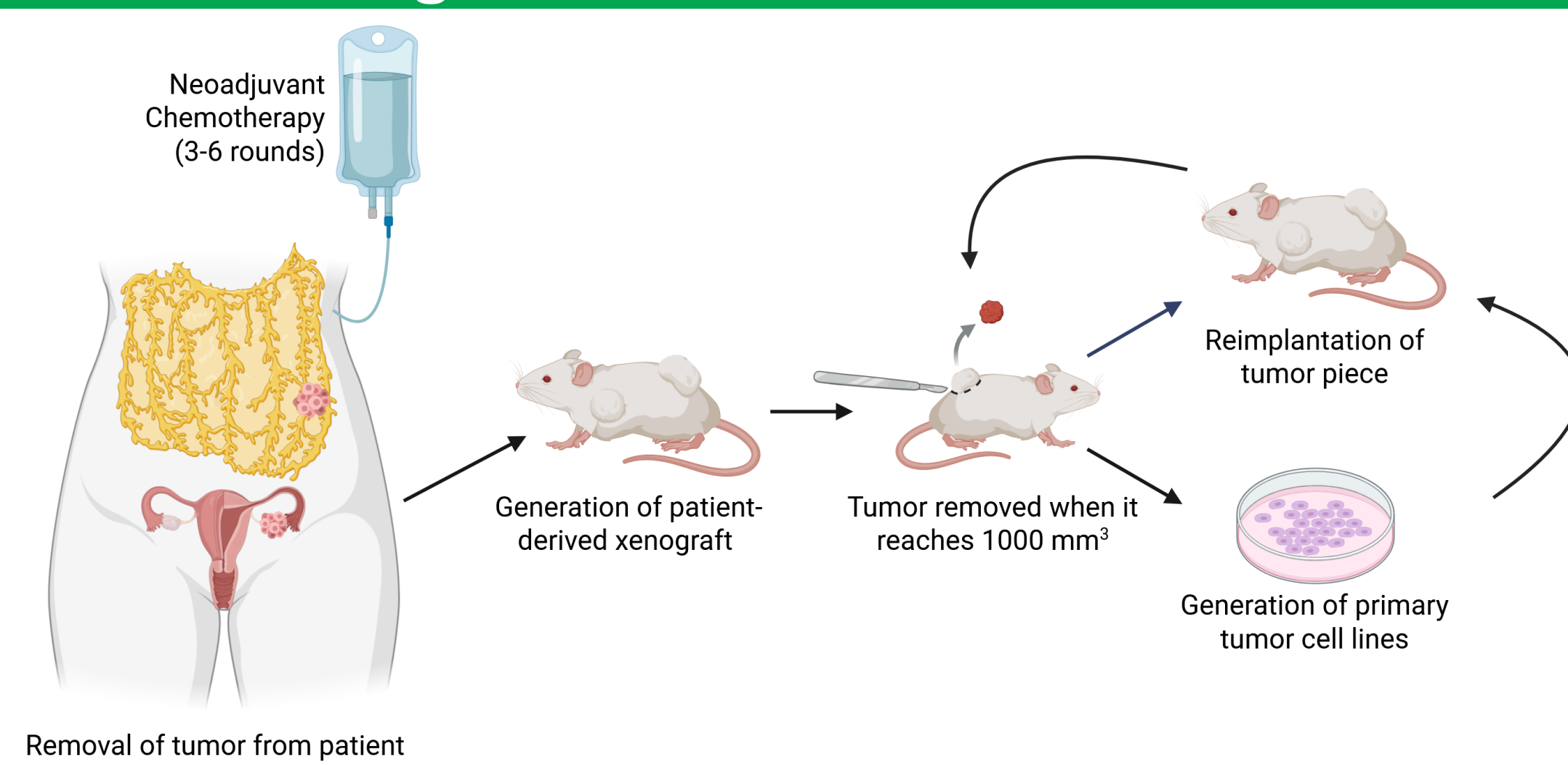


Fig. 1. Establishment of a persister ovarian cancer model. Primary high-grade serous ovarian tumor tissue was surgically resected from a patient at the University of Michigan and implanted into immunocompromised mice to generate patient-derived xenografts (PDXs). Following in vivo tumor growth, xenografted tumors were harvested and dissociated for ex vivo culture to establish patient-derived cell lines. Created using BioRender.com

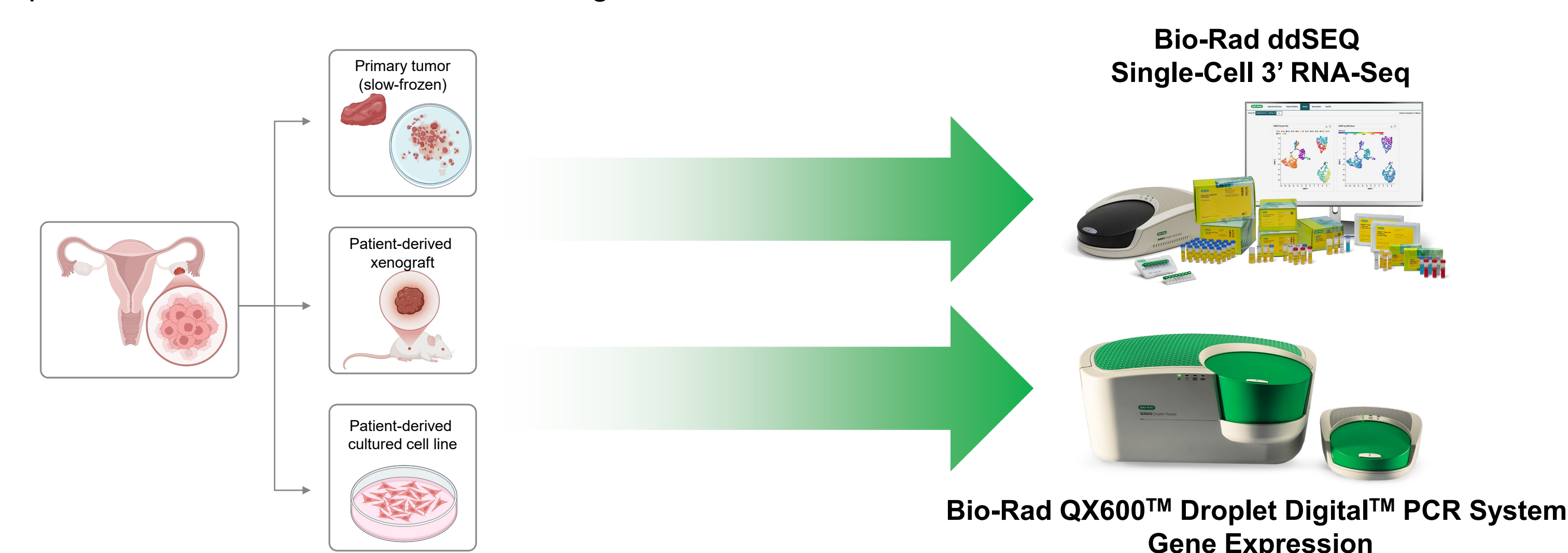


Fig. 2. Transcriptomic profiling of ovarian cancer models using ddSEQ Single-Cell RNA-Seq with ddPCR™ validation. Ovarian cancer models were analyzed using ddSEQ™ Single-Cell 3' RNA-Seq to profile gene expression at single-cell resolution and identify transcriptionally distinct cell populations. Differentially expressed genes from single-cell analyses were validated by absolute transcript quantification using the QX600™ ddPCR Gene Expression system, integrating high-throughput discovery with sensitive orthogonal validation. Partially created using BioRender.com.

Results

Single-Cell Transcriptomic Profiling of Ovarian Cancer Models

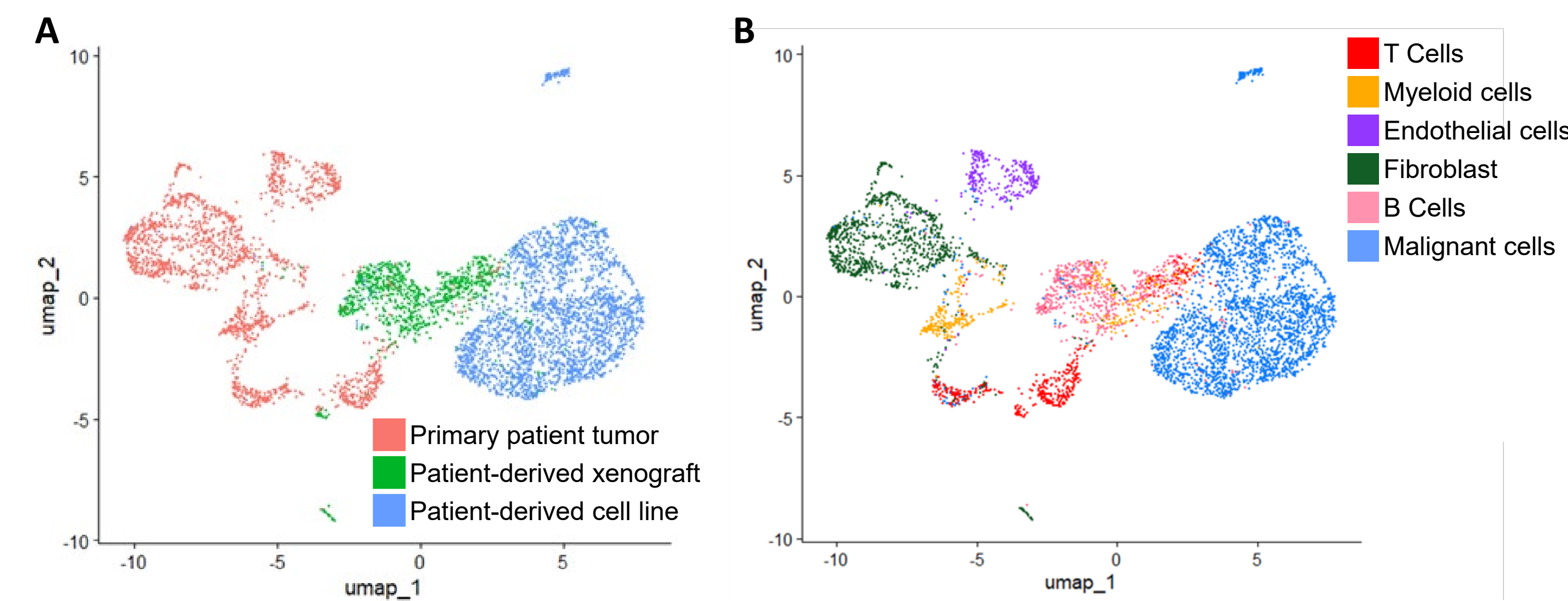


Fig. 3. Single-cell transcriptomics of ovarian cancer models. Uniform Manifold Approximation and Projection (UMAP) of three integrated ovarian cancer models classified based on (A) cancer model and (B) cell type classification, where each dot represents a single cell.

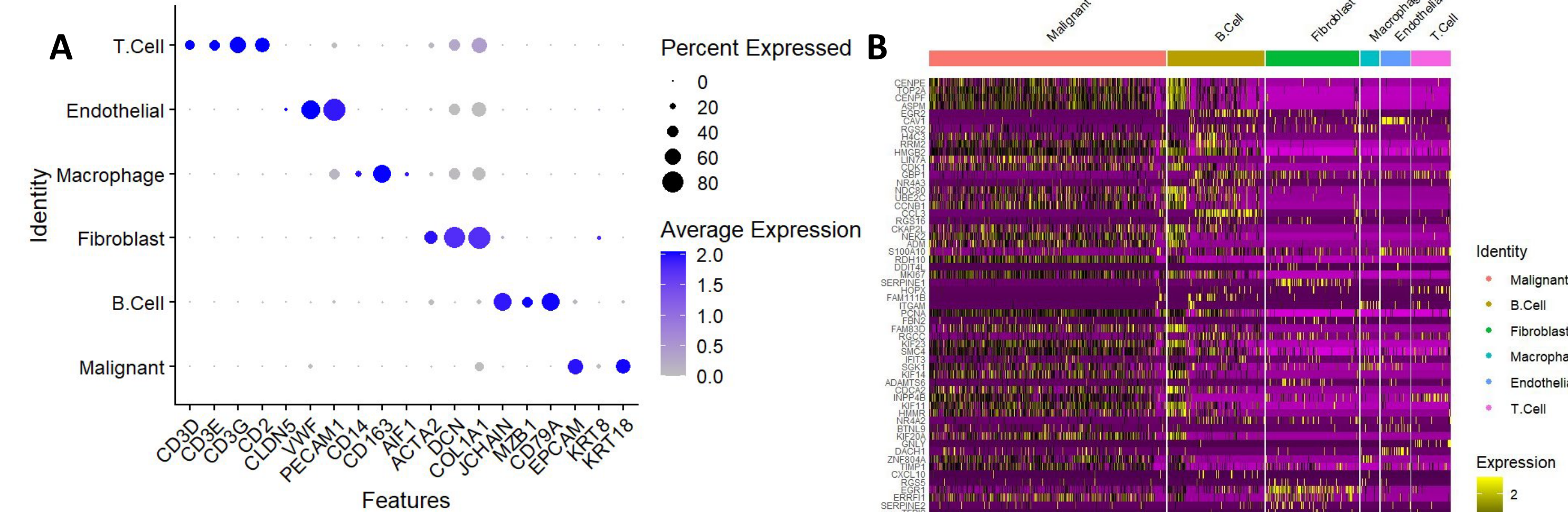


Fig. 4. Cell type-specific gene expression signatures across ovarian cancer model systems. (A) Dot plot and (B) heat map visualization depict the top differentially expressed genes for each cell type identified across patient-derived ovarian cancer models, showing increased expression of ovarian cancer markers, EpCAM and KRT18, in malignant cell type.

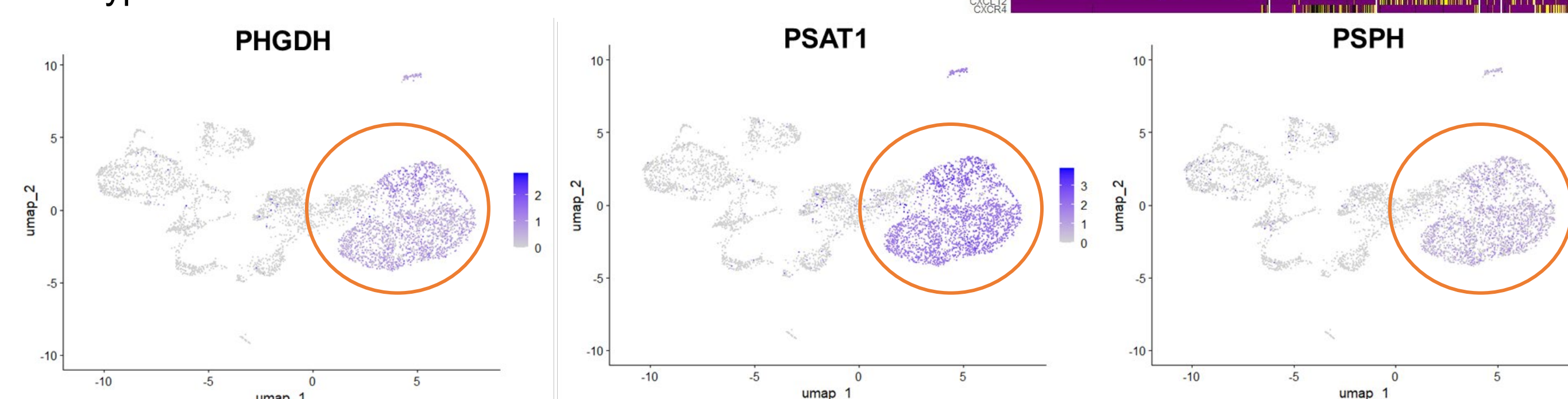


Fig. 5. Increased expression of genes associated with serine metabolism pathway in patient-derived cell lines. Uniform Manifold Approximation and Projection (UMAP) of integrated ovarian cancer models showing expression of PSAT1, PHGDH, PSPH – three genes associated with serine metabolism pathway.

Gene Expression Analysis Using ddPCR

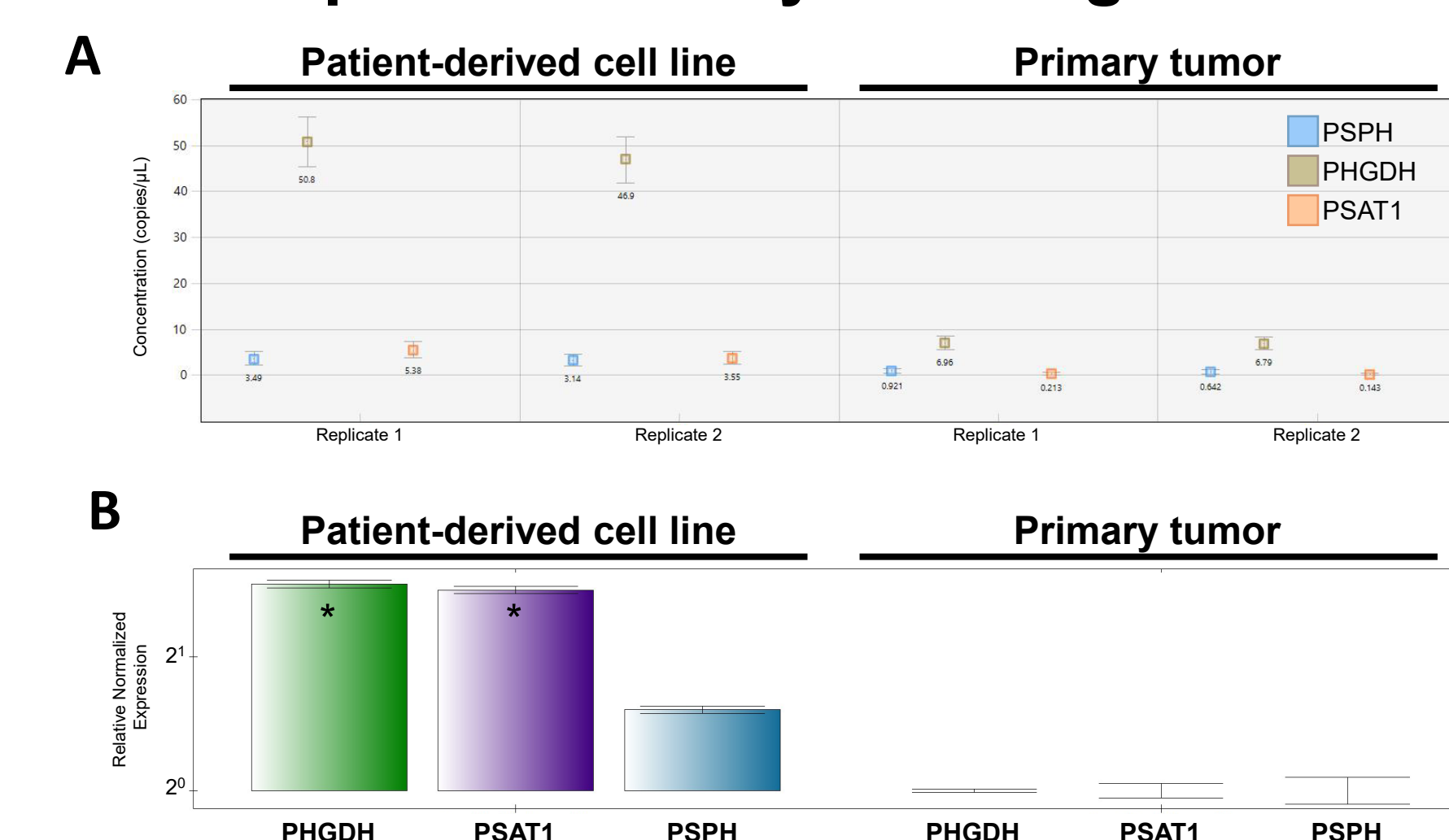


Fig. 6. Gene expression analysis of serine metabolism associated genes using multiplex ddPCR assay. (A) Dot plots showing concentrations for PSPH, PHGDH, and PSAT1 in copies/μL. (B) Box plots showing upregulation of serine metabolism-associated gene in patient-derived cell line vs primary tumor.

Materials and Methods

Generation of Ovarian Cancer Models

Primary ovarian tumor specimens were obtained from patients at the University of Michigan under Institutional Review Board (IRB)-approved protocols and in accordance with informed consent.

Primary Tumor Tissue: Primary tumor samples were processed shortly after surgical resection and cryopreserved using a controlled slow-freezing method in culture media + DMSO.

Patient-Derived Xenograft (PDX) Models: To establish PDX model, freshly resected primary tumor tissue was implanted into immunodeficient mice. Tumor growth was monitored, and xenografts were harvested once tumors reached approximately 1,000 mm³. Excised tumors were serially passaged into additional recipient mice to enrich for aggressive tumor cell populations. Multiple rounds of xenograft passaging were performed as part of model establishment.

Patient-Derived Cultured Cell Lines: Patient-derived cultured cell lines were generated from excised xenograft tumors. Harvested PDX tissue was dissociated and cultured under appropriate *in vitro* conditions to establish stable tumor cell lines for downstream experimental applications.

ddSEQ Single-Cell 3' RNA-Seq

Primary patient tumors were slow-frozen prior to dissociation and nuclei isolation, while PDX and cell line samples were prepared as high-viability (>90%) single-cell suspensions. Single-cell and single-nuclei suspensions were processed using the Bio-Rad ddSEQ Single-Cell 3' RNA-Seq workflow. Libraries were sequenced on an Illumina NextSeq 2000, and FASTQ files were analyzed using Omnitron Analysis Software (v1.1.0). The following settings were applied: *intron off* for cell input and *intron on* for nuclei input. Tertiary analyses were performed using the Seurat package (v5.3.0).

ddPCR System Gene Expression Analysis

Single-cell and single-nuclei suspensions prepared for scRNA-Seq were cryopreserved in RNeasy Lysis Buffer. RNA were isolated from cryopreserved samples using Qiagen RNeasy Micro Kit. Purified RNA (1 ng) was reverse-transcribed and amplified using iScript Explore One-Step RT and PreAmp Kit (Bio-Rad). cDNA was used for gene expression assay using Bio-Rad QX600™ Droplet Digital™ PCR System.

Gene ID	PrimePCR Assay	Fluorophore
PSAT1	dHsaCPE15065749	Cy5.5
PHGDH	dHsaCPE5033345	HEX
PSPH	dHsaCPE5044888	FAM
ACTB	dHsaCPE30032878	Cy5
GAPDH	dHsaGEXS440249864	ROX

QX Manager Gene Study software was used to estimate the target gene expression variations PSAT1, PHGDH, and PSPH between patient-derived cultured cell line and primary tumor as control and normalized with the internal reference gene (ACTB and GAPDH) copy number. Genes with significant fold changes (*p < 0.05) were considered differentially expressed.

Discussion and Conclusion

Using ddSEQ Single-Cell 3' RNA-Seq, we have shown that a diverse tumor microenvironment is present in HGSC that helps to maintain persister cells, including fibroblasts, macrophages, and endothelial cells. Furthermore, we found that the serine synthesis pathway is upregulated in the persister cell line, results that have been validated by ddPCR system gene expression analysis. Increased expression of serine synthesis genes is known to have tumorigenic effects. Serine is a nonessential amino acid and, as such, cells can either rely on exogenous sources of serine to survive or synthesize it directly. Highly proliferative cancer cells undergo metabolic reprogramming, a hallmark of malignant tumor progression, to increase de novo serine synthesis, thereby meeting the increased demand for amino acids and other downstream products.^{3,4} Metabolic reprogramming can also occur after chemotherapy treatment, such as the neoadjuvant chemotherapy majority of HGSC patients undergo. Additionally, increased serine synthesis can enhance cancer stemness, epithelial-mesenchymal transition (EMT), metastasis, and drug resistance in a wide range of cancers.⁵ The role of serine synthesis in ovarian cancer has been partially explored. In patients with epithelial ovarian cancer, the subtype HGSC belongs to, higher expression of PSAT1 is associated with significantly worse progression-free survival and overall survival.⁵ Further research is needed to fully understand how metabolic remodeling in TIC drives resistance to therapies, including platinum and immune checkpoint inhibitors.

References

- Lisio, M.-A., Fu, L., Goyeneche, A., Gao, Z. & Telleria, C. High-Grade Serous Ovarian Cancer: Basic Sciences, Clinical and Therapeutic Standpoints. *Int. J. Mol. Sci.* **20**, 952 (2019).
- Belur Nagaraj, A. et al. A miRNA-Mediated Approach to Dissect the Complexity of Tumor-Initiating Cell Function and Identify miRNA-Targeting Drugs. *Stem Cell Rep.* **12**, 122–134 (2019).
- Faubert, B., Solmonson, A. & DeBerardinis, R. J. Metabolic reprogramming and cancer progression. *Science* **368**, eaaw5473 (2020).
- Mattaini, K. R., Sullivan, M. R. & Vander Heiden, M. G. The importance of serine metabolism in cancer. *J. Cell Biol.* **214**, 249–257 (2016).
- Zhang, Y. et al. PSAT1 Regulated Oxidation-Reduction Balance Affects the Growth and Prognosis of Epithelial Ovarian Cancer. *OncoTargets Ther.* **13**, 5443–5453 (2020).

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