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Research Report

Molecular characterization of ovarian mesonephric-like adenocarcinoma: Insights from single-cell RNA sequencing and mitochondrial metabolism

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ABSTRACT

Objectives: Ovarian mesonephric-like adenocarcinoma (MLA) is a rare malignancy with limited understanding of its molecular features and therapeutic vulnerabilities. Although similar to uterine MLA, its unique characteristics remain undefined. This study aimed to characterize ovarian MLA using single-cell RNA sequencing (scRNA-seq) and compare it with high-grade serous ovarian cancer (HGSOC).
Methods: We analyzed the cellular and molecular heterogeneity of an ovarian MLA sample using scRNA-seq. Differential gene expression and pathway analyses were performed to identify unique molecular signatures and therapeutic targets. HGSOC scRNA-seq datasets were used for comparative analysis.
Results: Ovarian MLA demonstrated reduced heterogeneity, with a predominance of epithelial cells compared to HGSOC. Transcriptomic profiling revealed an upregulation of mitochondrial metabolism and lipid biosynthesis genes, indicating a metabolic shift toward oxidative phosphorylation. Gene enrichment and protein–protein interaction analyses identified distinct pathways, including mitochondrial biogenesis and dynamics, suggesting mitochondrial reprogramming.
Conclusions: This study provides the first scRNA-seq-based molecular characterization of ovarian MLA, differentiating it from HGSOC. Findings suggest potential therapeutic avenues, with a proposed combination therapy targeting MAP kinase and PI3K/AKT/mTOR pathways. Validation in larger cohorts is necessary for clinical application.

1. Introduction

Mesonephric adenocarcinoma (MA), a rare malignant neoplasm originating from the mesonephric duct remnants, primarily affects the cervix (Mirkovic et al., 2023). These tumors are distinguished by diverse histological patterns, including ductal, tubular, papillary, cribriform, retiform, sex cord-like, glomeruloid, sieve-like, spindle, and solid forms (Mirkovic, Olkhov-Mitsel, 2023). Immunophenotypically, MAs exhibit variability but typically express paired box protein 8 (PAX8), GATA binding protein 3 (GATA3), thyroid transcription factor 1 (TTF1), and CD10 (luminal staining) and not estrogen receptor (ER) and progesterone receptor (PR). They are also characterized by molecular features, such as *KRAS* and/or *NRAS* mutations and frequent gain of chromosome 1q (Jia et al., 2019).

In 2016, the term "mesonephric-like adenocarcinoma" (MLA) was introduced for a previously undescribed type of MA arising in the uterine corpus, notably in the ovaries (McCluggage, 2022). MLAs exhibit both morphological and immunophenotypical similarities to MAs but are

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distinct, especially because they occur in the ovary, which is extremely rare (Pors et al., 2021). Ovarian MLAs often present mixed growth patterns and luminal eosinophilic secretions. Their nuclei are hyperchromatic and pleomorphic and at times resemble those of papillary thyroid carcinomas. Molecularly, they share similarities with MAs but remain unexplored owing to their rarity (Jia, Sun, 2019).

The scarcity of data on ovarian MLAs and their recent inclusion in the 2020 World Health Organization (WHO) classification of female genital tumors highlight the need for a more focused research (McCluggage et al., 2022). The potential for misdiagnosis is high as it can be mistaken for other Müllerian carcinomas with unusual morphologies and immunophenotypes. Given the lack of detailed molecular understanding and limited number of cases with adequate clinical follow-up, predicting the overall prognosis of ovarian MLAs is difficult (McCluggage, 2022). The most common post-operative treatment involves a combination of carboplatin and paclitaxel; however, the clinical response to this regimen is variable and many patients show disease progression (McCluggage, 2022).

Technological advances have significantly improved gene expression analysis, enabling high-resolution studies at the single-cell level (Tang et al., 2009). Single-cell RNA sequencing (scRNA-seq) offers a profound understanding of cellular diversity and heterogeneity, crucial for uncovering the mechanisms of oncogenesis and paving the way for personalized therapies (Tang, Barbacioru, 2009). Therefore, in the present study, we applied scRNA-seq to investigate the unique molecular features of ovarian MLAs, compared with those of high-grade serous ovarian cancer (HGSOC). Our objectives are to: (i) delineate the cellular heterogeneity of ovarian MLAs, (ii) identify distinct molecular signatures specific to ovarian MLAs, and (iii) explore potential therapeutic targets for ovarian MLAs. This comprehensive approach aimed to enhance our understanding of the pathogenesis of ovarian MLA and inform future clinical applications, potentially transforming the management and treatment of this rare and challenging malignancy.

2. Methods

2.1. Patient and tumor sample

We collected the ovarian MLA sample during debulking surgery at the Gangnam Severance Hospital, Seoul, Korea. Fresh tissues were immediately processed to ensure optimal preservation. The tissue was dissected into fractions for enzymatic digestion into single cells and fixed in 4 % paraformaldehyde solution, followed by paraffin embedding.

Ovarian MLA tissue was histologically confirmed by a gynecology specialized pathologist using the International Federation of Gynecology and Obstetrics (FIGO) and WHO grading systems. Clinical information, including chemotherapy history, was retrieved from the patient's medical records. Ethical compliance was ensured through approval by the Institutional Review Board of Gangnam Severance Hospital (IRB No. 3-2023-0138) and all methods were performed in accordance with relevant guidelines and regulations. Due to the retrospective design of the study, informed consent was waived by the IRB.

2.2. Acquisition of public data

Public scRNA-seq data for HGSOC were obtained from the the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) database, specifically from the datasets GSE 184880(Xu et al., 2022), GSE191301 (Shen et al., 2022), and GSE154600(Wang et al., 2023). Further detailed information about each data were provided in Supplementary Data 1.

2.3. Immunohistochemical staining

Briefly, 4-μm-thick unstained sections obtained from formalin-fixed paraffin-embedded tissue from surgically resected ovary were subjected to hematoxylin and eosin staining using the Tissue-Tek Prisma Plus automated slide stainer (Sakura Finetek). Immunohistochemical staining for PAX8 (mouse monoclonal, 363 M−16, 1:70, Cell marque, United States) and ER (mouse monoclonal, NCL-L-ER-6F11, 1:400, Novocastra, United Kingdom) was performed using the BOND-MAX fully automated staining system (Leica Biosystems), and that for GATA3 (mouse monoclonal, CM405A, 1:200, Biocare, United States) and TTF-1 (mouse monoclonal, M3575, 1:100, Dako, Denmark) was performed using the Ventana BenchMark XT automated platform (Ventana Medical Systems).

2.4. Tissue dissociation and single cell preparation

Fresh specimens were immediately stored in Tissue Storage Solution (Miltenyi Biotech, cat no. 130–100-008) at 4 °C. Prior to dissociation, tissues were washes with phosphate-buffered saline (PBS) and minced into 1–2-mm pieces. Tissues were dissociated using the Tumor Dissociation Kit, human (Miltenyi Biotech, cat no. 130–095-929) and gentleMACS™ Dissociator (Miltenyi Biotech), following the manufacturer's instructions. The resulting cell suspension was filtered, centrifuged, and erythrocytes were lysed. Cells were washed counted and assessed for viability before dead cell removal. Detailed protocols were provided in Supplementary Data 2.

2.5. Single cell 5′ gene expression library construction and sequencing

According to the 10x Chromium Single Cell 5′ v2 protocol (10x Genomics, document no. CG000331_ChromiumNextGEMSingleCell5-v2_UserGuide_RevE), single cell RNA-seq library was prepared using the 10x Chromium controller and Next Gem Single cell 5′ Reagent v2 kits (10x genomics, PN-1000264). The detailed information on the library construction and sequencing were provided in supplementary Data 3.

2.6. Single-cell RNA data processing and identification of cell types

scRNA-seq datasets were analyzed using the Seurat package in R 4.2.2 (Stuart et al., 2019). We filtered cells based on RNA content and mitochondrial RNA (<50000 of the nCount_RNA, <7500 of the nFeature_RNA, and < 20 % of the mitochondrial genes expressed), annotated cell types by well-established marker genes list provided in supplementary Data 2, alongside established marker genes from prior studies and further details were provided in supplementary Data 4 (Xu, Fang, 2022; Shen, Ren, 2022; Ren et al., 2022; Wan et al., 2021; Nath et al., 2021; Shih et al., 2018).

2.7. Pathway analysis

Using the FindAllMarkers function in the Seurat package, we analyzed the $\log_2$ fold changes in differentially expressed genes (DEGs) between HGSOC and ovarian MLA cells, applying thresholds of (i) LogFC > 0.3, (ii) $P < 0.05$, and (iii) min.PCT > 0.25. The identified DEGs were then visualized on a UMAP and further scrutinized using violin and feature plots for expression patterns. Protein–protein interaction (PPI) was explored using the STRING database to understand the DEGs' biological implications and Gene Set Enrichment Analysis (GSEA) with gProfiler was conducted on the genes presented in the PPI network (Rath et al., 2021; Szklarczyk et al., 2015; Kolberg et al., 2023; Korotkevich et al., 2021). A focus on mitochondrial genes from MitoCarta3.0 provided insights into the affected biological pathways, applying GSEA with Fast Gene Set Enrichment Analysis (FGSEA). To ensure enough DEGs for downstream analyses, DEGs were selected based on the *P*-value rather than the adjusted *P*-value, given the significant discrepancies between the two.

2.8. Statistical analysis

Student's *t* test was used to analyze continuous variables. The Kruskal–Wallis test was used for comparisons among three or more groups. Statistical analyses were conducted using R version 4.2.2, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Case presentation and histopathology results

A 71-year-old female with an incidental finding of a right ovarian cyst during a routine health checkup was evaluated at our hospital. Imaging studies included the following: abdominal computed tomography (CT) scan, which identified a 7.4-cm cystic mass in the right ovary (Fig. 1a); magnetic resonance imaging with T2-weighted sequences, which characterized the mass as an 8.4-cm mixed cystic and solid lesion suggesting epithelial ovarian cancer (Fig. 1b and 1c); and positron emission tomography-computed tomography (PET-CT) scanning, which demonstrated increased fluorodeoxyglucose uptake in the solid portion of the ovarian mass, indicating malignancy, with no significant uptake in the lymph nodes or distal sites (Fig. 1d). The levels of tumor markers were notably elevated, with a CA 125 level of 105 U/ml (normal range: 0–35 U/ml) and CA19-9 level of 1380 U/ml (normal range: 0–37 U/ml), further supporting the diagnosis of the malignancy. The patient underwent a total abdominal hysterectomy, bilateral salpingo-oophorectomy, pelvic lymphadenectomy, infracolic omentectomy, and peritoneal cytology. Pathological examination post-surgery classified the tumor as stage IA based on the 2014 FIGO classification. Following the surgery, she completed cycles of taxane- and platinum-based chemotherapy regimens. At the 12-month follow-up, the patient exhibited no signs of disease, confirming a period of disease-free survival.

The excised specimen was a complex cystic and solid mass with friable and segmented tissues (Fig. 1e). Histopathological assessment at varying magnifications depicted a predominantly tubular architecture with eosinophilic colloid-like material within the tubular lumen, aligning with the characteristics of ovarian MLA (Fig. 1f, 1 g and 1 h). Moreover, immunohistochemical analysis revealed diffuse and strong positive immunoreactivity for PAX8 (Fig. 1i) and GATA3 (Fig. 1j), focal positivity for TTF-1 (Fig. 1k), and negativity for ER (Fig. 1l).

3.2. Comparative cellular heterogeneity analysis of ovarian mesonephric-like adenocarcinomas and HGSOC

In our comparative analysis using scRNA-seq dataset (GSE184880, GSE191301, and GSE154600), we observed a distinct cellular composition in ovarian MLA compared to that in HGSOC, the most common subtype of ovarian malignancy (Webb and Jordan, 2017). Initial observations using UMAP clustering suggested that ovarian MLA displayed a lesser degree of cellular heterogeneity compared than HGSOCs (Fig. 2a). After subsequent annotation of cell phenotypes, we observed a notably higher proportion of cancer epithelia l cells in ovarian MLA (78 %) than in HGSOCs, in which the proportion of cancer epithelial cells ranged from 14.6 % to 31.4 % across datasets (Fig. 2b and c). Moreover, the cellular composition of ovarian MLA was characterized by the presence of six distinct cell types, cancer epithelial cells, endothelial cells, fibroblast/stromal cells, monocytes/macrophages, NK/T cells, and myeloid cells, with a conspicuous absence of B cells. In contrast, B cells were quantified at 2.0 %, 5.6 %, and 6.2 % within HGSOCs (Fig. 2d). The differential expression profiles of B cell marker genes in both ovarian MLA and HGSOCs are shown in Fig. 2e. Our findings provide a granular understanding of the cellular heterogeneity that distinguishes ovarian MLA from HGSOCs. The notable preponderance of cancer epithelial cells and absence of B cells in ovarian MLA underscore the potential pathophysiological mechanisms unique to this subtype, which may have substantive implications for the development of targeted treatment modalities.

3.3. Comparative gene expression patterns in epithelial, fibroblast/stromal, and immune cells across ovarian MLA and HGSOC: Focused gene expression profiling

Considering that ovarian MLA are relatively less heterogeneous than HGSOCs from a cell-type perspective, we searched for ovarian MLA-specific gene expression profiles to identify certain groups of genes that are directly or indirectly associated with ovarian MLA. As shown in Fig. 3a, the expression levels of selected gene group were notably higher in the cancer epithelial cells of ovarian MLA but barely detectable in HGSOC. To further validate the specificity of these for ovarian MLA, we compared expression levels across other cell types, such as fibroblast/stromal cells and immune cells. This comparison highlights that the selected genes are predominantly expressed in the epithelial cells of ovarian MLA, reinforcing their specificity to this cell type. To delve deeper into differential gene expression within epithelial cells, we analyzed the gene expression patterns using violin plots, as shown in Fig. 3b. Notably, the overexpression of KRAS was detected in ovarian MLA epithelial cells, which is recognized as a key oncogenic driver in this cancer type, independent of its cellular origin, when to HGSOC(Lin et al., 2020). In contrast, NRAS was not overexpressed in ovarian MLA compared to HGSOC. Additionally, several other genes, including 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2), chromogranin A (CHGA), and phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 gamma (PIK3C2G), exhibited pronounced association with ovarian MLA (Supplementary Table 1).

The number of cells in fibroblast/stromal cells, immune cells, and epithelial cells of Ovarian MLA were 2,154, 1,186, and 17,179. In GSE154600 samples, there were 5,056 cells in fibroblast/stromal cells, 27,879 in immune cells, and 15,406 in epithelial cells. In GSE191301 samples, there were 300 cells in fibroblast/stromal cells, 13,871 cells in immune cells, and 2,435 cells in epithelial cells. Lastly, in GSE184600, there were 2,236 cells in fibroblast/stromal cells, 20,400 cells in immune cells, and 4,676 cells in epithelial cells. **(b)** Violin plots displaying the gene expression levels in epithelial cells in both ovarian mesonephric-like adenocarcinoma and high-grade serous ovarian cancer. HGSOC: High-grade serous ovarian cancer; Ovarian MLA: Ovarian mesonephric-like adenocarcinoma.

3.4. Network pathway analysis and mitochondrial function divergence in ovarian MLA versus HGSOC: Gene interaction, enriched pathways, and metabolic profiling

Having found the transcriptomic profiles of the ovarian MLA-specific gene group and recognizing that none of the genes were functionally associated with each other as a whole, we examined their association through PPIs, as shown in Fig. 4a (Supplementary Table 2). Two PPI-based clusters were identified with relatively high confidence levels, where four and two proteins were strongly correlated. One included GATA3, transforming growth factor beta1, CHGA, and POU class 3 homeobox 3, and the other included HMGCS2 and cytochrome P450 family 51 subfamily A member 1. The larger cluster revealed associations with known functions, such as HMG box domain binding, positive regulation of epithelial morphogenesis, cell migration, macrophage differentiation, ureteric bud morphogenesis, mammary gland epithelium development, cellular response to ionizing radiation, cell motility, mesonephric tubule morphogenesis, and regulation of epithelial morphogenesis. Based on previous studies, it is probable that the HMG box domain may have a potential link with ovarian MLA-specific functional mechanisms (Grimm et al., 2020; Castillo et al., 2012). The other cluster presented enriched functions, such as sterol 14-demethylase activity, cholesterol biosynthetic/metabolic processes, steroid biosynthetic processes, sterol metabolic processes, alcohol biosynthetic processes, and secondary alcohol biosynthetic/metabolic processes. Additionally, we performed GSEA

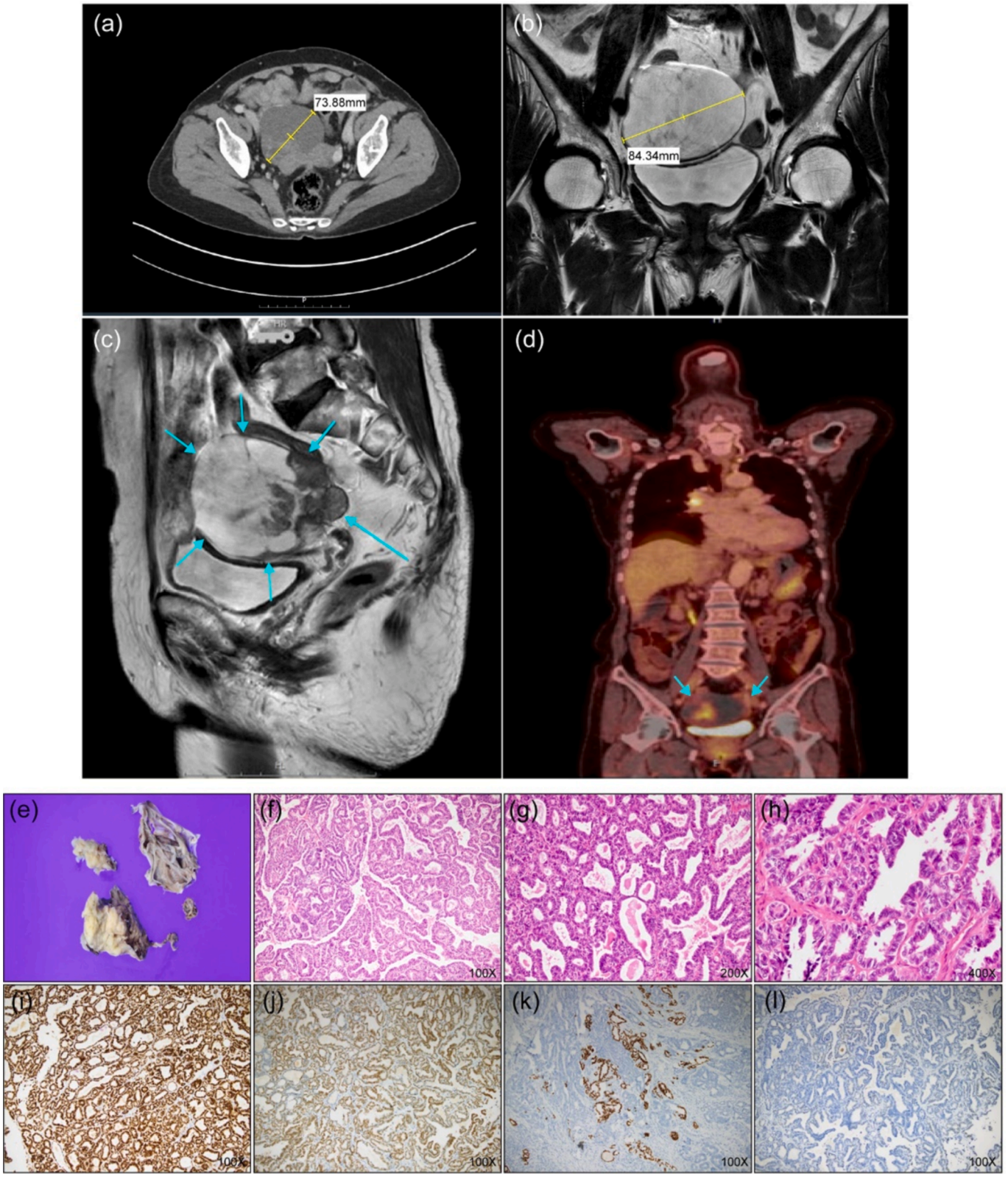


Fig. 1. Imaging, gross and histological image of ovarian mesonephric-like adenocarcinoma. **(a)** Axial T1-weighted pelvic magnetic resonance image of right adnexa. **(b)** Coronal T2-weighted magnetic resonance image of right adnexa. **(c)** Sagittal T2-weighted magnetic resonance image of right adnexa. **(d)** Positron emission tomography-computed tomography scan with increased metabolic activity in right adnexa. **(e)** Gross pathology specimen exhibiting the resected mass. **(f–h)** Histological sections of mass stained with hematoxylin and eosin showing different magnifications (100 × to 400 ×). Immunostaining of **(i)** PAX-8, **(j)** GATA-3, **(k)** TTF-1, and **(l)** ER.

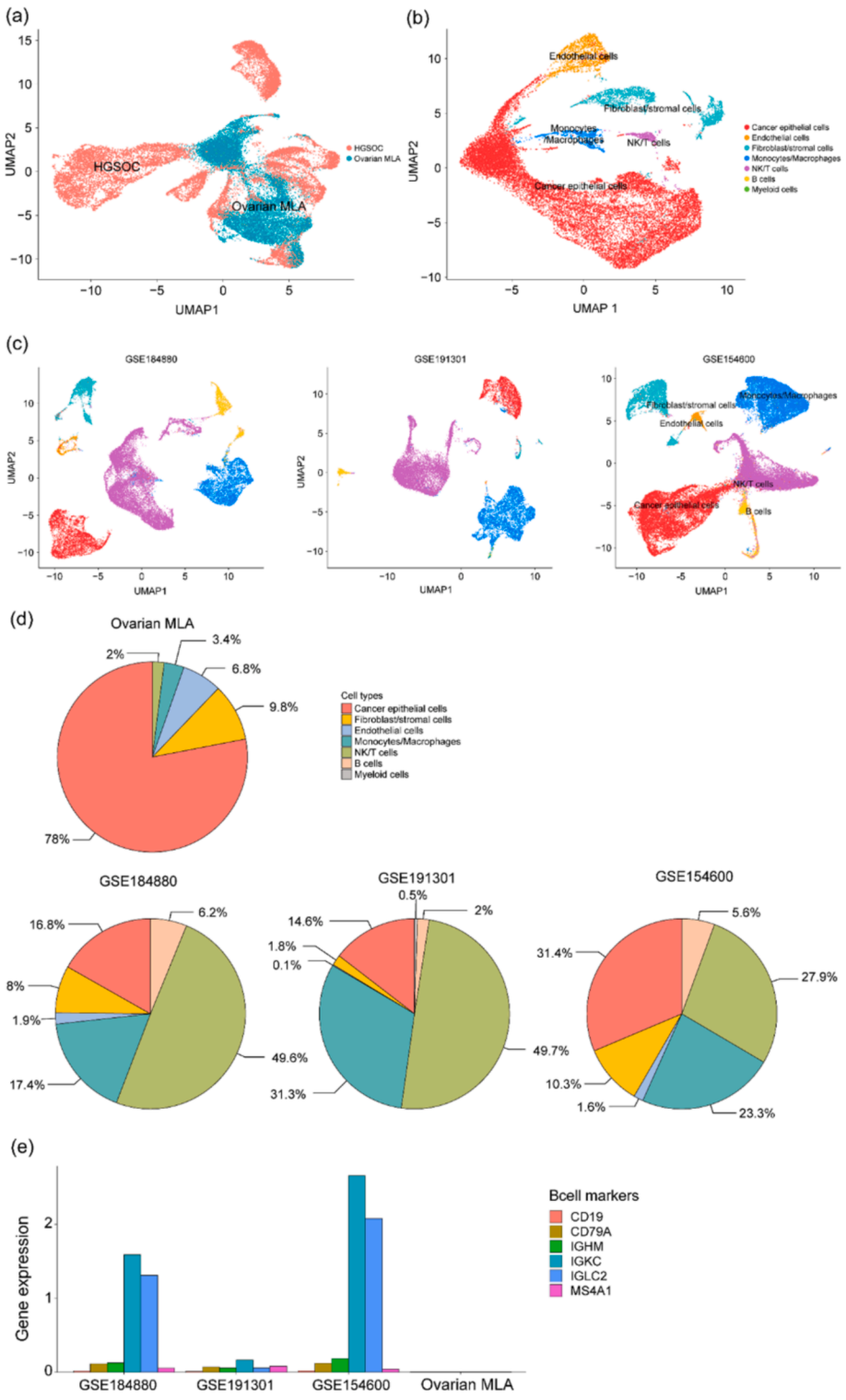


Fig. 2. UMAP of ovarian mesonephric-like adenocarcinoma and high-grade serous ovarian cancer and cell type proportions **(a)** UMAP of the integrated epithelial cells in ovarian mesonephric-like adenocarcinoma and three high-grade serous ovarian cancer datasets. **(b)** UMAP of ovarian mesonephric-like adenocarcinoma. **(c)** UMAP of high-grade serous ovarian cancer separately (GSE184880, GSE191301, and GSE154600). **(d)** Pie charts showing the proportions of cell types in ovarian mesonephric-like adenocarcinoma and three high-grade serous ovarian cancer revealing high expression of cancer epithelial cells in ovarian mesonephric-like adenocarcinoma. **(e)** Gene expression levels of B cell markers across different datasets, including GSE184880, GSE191301, and GSE154600 and ovarian MLA. HGSOC: High-grade serous ovarian cancer; Ovarian MLA: Ovarian mesonephric-like adenocarcinoma.

using FGSEA to compute the up/down regulation patterns of mitochondria-associated pathways that can indirectly estimate alterations in mitochondrial energy metabolism (Fig. 4b, Supplementary Table 3). Ovarian MLA displayed a significantly higher positive enrichment in the mitochondrial metabolism, dynamics and surveillance than HGSOCs. Mt-tRNA synthetases for the mitochondrial energy metabolism and MICOS complex for mitochondrial dynamics and surveillance were specifically upregulated in ovarian MLA. Regarding

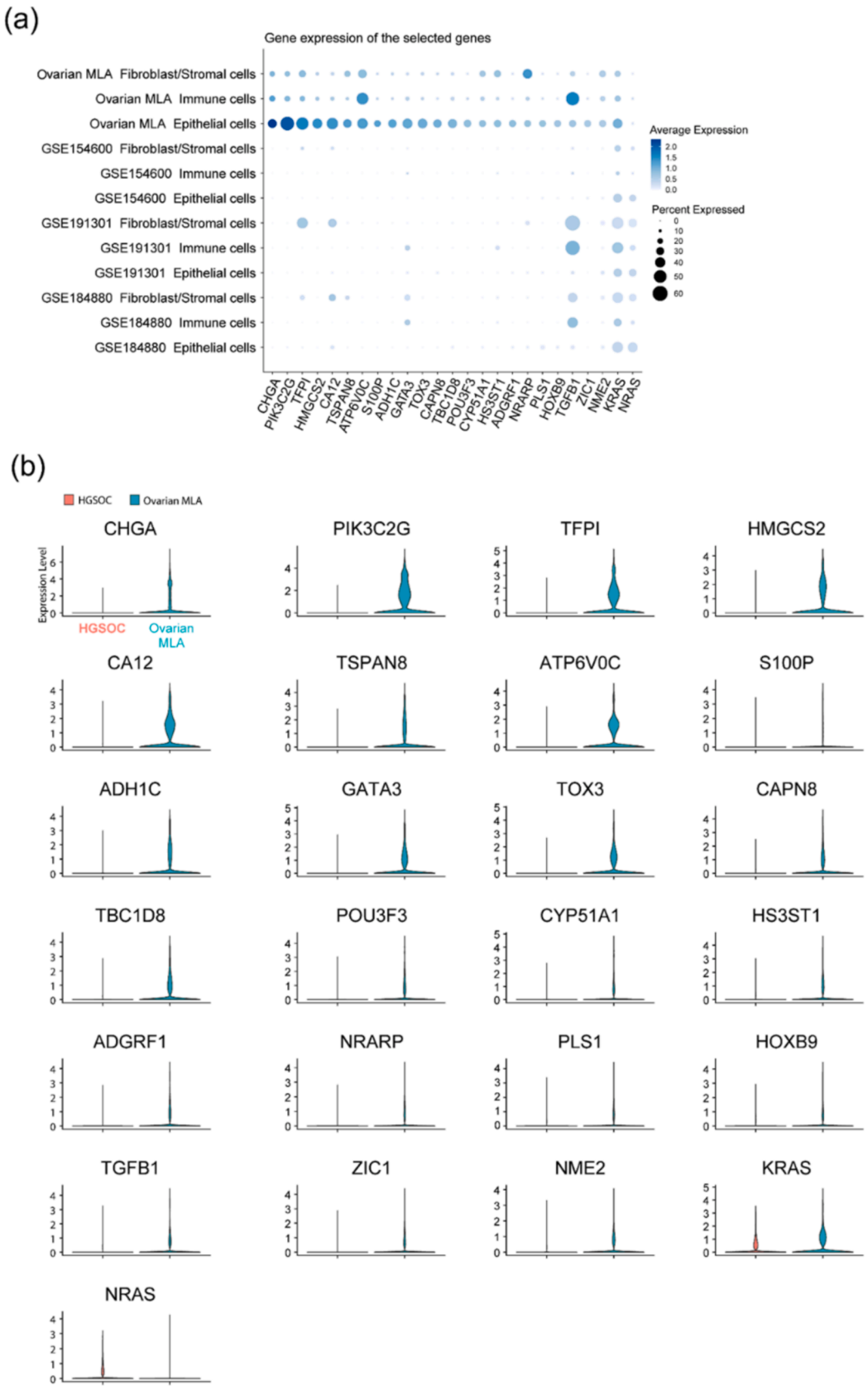


Fig. 3. Expression of the selected genes. **(a)** Expression of the selected genes in epithelial cells, fibroblast/stromal cells, and immune cells.

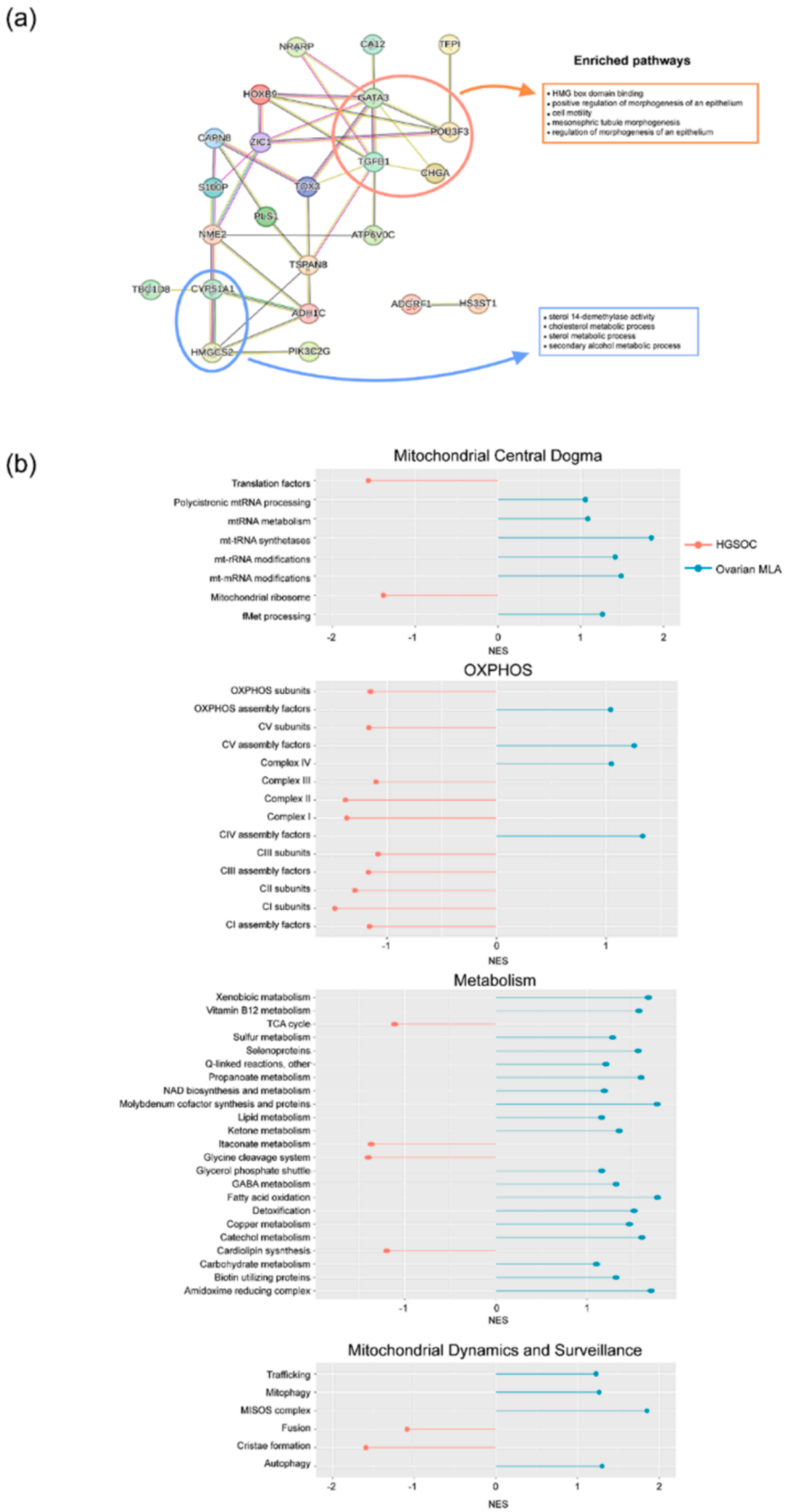


Fig. 4. Protein interaction network and mitochondrial analysis in ovarian mesonephric-like and high-grade serous ovarian cancer **(a)** Protein–protein interaction network of proteins encoded by the selected genes, along with enriched pathways identified by GSEA utilizing gProfiler within high confidence clusters. **(b)** Enriched pathways of mitochondrial central dogma, oxidative Phosphorylation (OxPhos), metabolism, and mitochondrial dynamics and surveillance, comparing ovarian mesonephric-like adenocarcinoma and high-grade serous ovarian cancer by MitoCarta3.0. Blue lollipop indicates upregulation in ovarian MLA, and pink indicates upregulation in HGSOC. HGSOC: High-grade serous ovarian cancer; Ovarian MLA: Ovarian mesonephric-like adenocarcinoma.

metabolic alterations, several upregulations in ovarian MLA were estimated for fatty acid oxidation, amidoxime-reducing complex, xenobiotic metabolism, catechol metabolism, propanoate metabolism, vitamin B12 metabolism, selenoproteins, and detoxification pathways. Oxidative phosphorylation also showed a dominant elevation of pathways (i.e., CI subunits, complex I, and complex II).

4. Discussion

In the present study, we present the first scRNA-seq analysis of ovarian MLA, uncovering their unique molecular characteristics, which are distinct from those of HGSOC. Our investigation of the tumor microenvironment at the single-cell level revealed a distinct metabolic phenotype in ovarian MLA characterized by increased mitochondrial activity, marking a departure from established cancer metabolism paradigms, such as the Warburg effect. Research regarding these unexplored aspects will aid the identification of several potential therapeutic targets, which, in turn, will offer novel insights into the pathogenesis of ovarian MLA and aid the development of future treatment approaches.

Our study provides insights into the molecular characteristics of ovarian MLA. Koh *et al.* suggested that the aggressive phenotype is characterized by mitotic activity and tumor necrosis (Koh et al., 2022). In the present study, we unveiled a notable enrichment of tumor epithelial cell clusters, demonstrating enhanced mitochondrial functionality within ovarian MLAs and providing novel insights into their metabolic adaptability, a hallmark of an aggressive cancer phenotype. We observed more dominant enrichment of epithelial cell clusters in ovarian MLA than in HGSOCs. Using focused GSEA, we identified key DEGs within the epithelial cluster, including KRAS, HMGCS2, PIK3C2G, and CHGA, to shed light on the metabolic programming of ovarian MLA (Qiu et al., 2024). Of particular importance, our findings revealed abnormal KRAS expression in ovarian MLA, corroborating its role as a pivotal oncogenic driver in mesonephric-like carcinomas originating from ovarian, endometrial, or cervical tissues. This is consistent with previous studies that have documented the molecular underpinning of MLAs (Mirkovic et al., 2015; Na and Kim, 2019; Deolet et al., 2021). The clinical significance of this discovery is underscored by the recent advances in KRAS-targeted therapies offering potential therapeutic avenues for patients with ovarian MLA, particularly those exhibiting KRAS-driven tumorigenesis (Garlich et al., 2008; Ray-Coquard et al., 2023). HMGCS2, a rate-limiting enzyme in ketone body synthesis and a crucial mitochondrial enzyme, has been implicated in various diseases, including colorectal cancer and hepatocellular carcinoma, and it plays a pivotal role in mitochondrial metabolic reprogramming (Li et al., 2021; Fang et al., 2021). CHGA, which is associated with poor prognosis in various cancers, including breast and ovarian cancers, contributes to the regulation of tumor vascular biology and mitochondrial biogenesis, thereby affecting tumor cell proliferation, invasion, trafficking, and metastasis (Crippa et al., 2013; Belloni et al., 2007). PIK3C2G, a gene that is upregulated in ovarian MLA, is also known for its role in regulating glycolysis and ATP production; it has been linked to the recurrence and overall survival of colorectal cancer patients, with mutations identified in uterine MLA (Kim et al., 2022). These findings suggest the potential involvement of PI3K family members in ovarian MLA. In summary, our study underscores the significance of epithelial cell clusters associated with cell proliferation and energy metabolism in ovarian MLA that exhibit elevated mitochondrial activation; this is consistent with our finding regarding the enrichment of pathways related to mitochondrial activity. This heightened energy demand is evident through upregulated oxidative phosphorylation and the tricarboxylic acid cycle, which is consistent with the metabolic profile of HGSOCs. Additionally, ovarian MLA showed positive enrichment in lipid metabolism, with an intriguing increase in mitochondrial RNA metabolism, mt-tRNA synthesis, and the numbers of mt-rRNA and mr-mRNA modifications and enhancement of mitophagy and autophagy, possibly indicating a recovery from mitochondrial degradation in ovarian MLA epithelial cells. Consistent with these findings, previous studies on non-small-cell lung cancer have identified a distinct epithelial cell subset with a high differentiation potential that is enriched in biological processes related to cell proliferation and energy metabolism. Most importantly, recent findings focusing on the Warburg effect explain that active mitochondrial function and cell differentiation can reduce cell-type evaluation, which is consistent with the findings of our study; however, the constitutive activation of intracellular kinase signaling may promote disease progression (Jia et al., 2018). These insights have enhanced our understanding of the unique biology of ovarian MLA.

In the case of ovarian MLAs, for which definitive treatment strategies are yet to be established owing to limited clinical insights and an incomplete understanding of their molecular underpinnings, our research offers a preliminary understanding that may inform future studies. By investigating the molecular landscape of ovarian MLA, we suggest a novel dual inhibitory strategy that combines cobimetinib, a MAP kinase pathway inhibitor, with SF1126, a vascular targeted pan PI3K inhibitor prodrug with antitumor and antiangiogenic activity (Garlich et al., 2008; Ray-Coquard, Pignata, 2023). We drew our hypothesis from previous studies, which reported the MAPK pathway as a well-known pathway involved in tumorigenesis, tumor progression, and drug resistance, especially in rare epithelial ovarian cancers (Ray-Coquard, Pignata, 2023). In addition, compelling clinical evidence from the BOUQUET trial has indicated that cobimenitib, a selective MEK1/MEK2 inhibitor, showed promising activity against ovarian MLA, with a disease control rate of 89 % in patients with recurrent ovarian MLA compared with that in patients with other rare types of epithelial ovarian cancer, such as clear-cell ovarian cancer or carcinosarcoma (ORR 16 %) (Ray-Coquard, Pignata, 2023). Furthermore, our findings on the overexpression of genes associated with the PI3K/AKT/mTOR pathway are related to mitochondrial metabolism and the ability of SF1126 to interfere with this pathway, thereby reducing glycolytic flux and tumor progression in various solid cancers. This represents a mechanism that is complementary to that underlying the action of cobimenitib action (van der Mijn et al., 2016; Qin et al., 2019). We propose that co-targeting these pathways could potentially disrupt the survival and proliferation of ovarian MLAs; this requires further experimental validation. The promise of this combination therapy should be underpinned by the preclinical evidence that the simultaneous inhibition of the activities of MEK and PI3K may yield enhanced antitumor effects. Thus, we advocate for clinical trials tailored to evaluate the efficacy and safety of the combination of cobimenitib and SF1126 in patients with ovarian MLAs, with the potential to substantially shift the current treatment paradigm toward a more personalized and effective approach.

Our findings also indicate a notable depletion of B cells in ovarian MLA compared to the case for HGSOCs. B cells, typically abundant in HGSOCs, play key roles in immunity and their function in cancer is debated (Macpherson et al., 2020). In some cancers, B cells may promote tumor growth and angiogenesis, while in others, their depletion has been associated with reduced tumor progression (Lundgren et al., 2016). Given the complexity of B cell roles in cancer, further research is essential to understand their specific impact on ovarian MLAs.

Our study has a few limitations. Due to the scarcity of archived tissue samples, owing to the extreme rarity of ovarian MLAs, which comprise less than 1 % of all gynecological malignancies, our analysis was limited to a single sample. This also means that the molecular characteristics of ovarian MLAs have not yet been fully classified. Future research should involve larger cohorts of ovarian MLA samples to validate these observations and employ comprehensive analytical methods. This includes inferring copy number variations and examining mutations in genes such as *KRAS* or *NRAS* from scRNA-seq data to obtain results with significant statistical power. In addition, the focus of the GSEA analysis on mitochondrial pathways and metabolism arises from their critical role in cancer drug research.

Despite the availability of various anticancer strategies, inhibiting the energy supply to cancer cells remains one of the most common approaches. This study hypothesized that the increased aggressiveness of MLA cancer cells compared to the Serous type may be due to a relatively greater or different type of energy supply. Consequently, the analysis concentrated on mitochondrial-associated pathways, which are pivotal in energy provision. However, this focus also presents a limitation as it does not provide a comprehensive overview of all potential pathways.

Despite these limitations, our study holds significant value as to the best of our knowledge, it represents the first scRNA-seq analysis of ovarian MLA, providing novel insights into its unique molecular characteristics, compared with those of HGSOC. This groundbreaking research highlights the potential for future studies to build upon our findings and advance the understanding of ovarian MLA. Therefore, future studies including large cohorts of ovarian MLA samples could enhance the statistical power and robustness of the findings. Integrating multi-omics approaches, such as proteomics and epigenomics approaches, also could provide a more comprehensive understanding of the molecular mechanisms underlying the occurrence of ovarian MLAs; this would enable the translation of such approaches and information into clinical practice and ultimately, drive a shift towards the development of more personalized and effective treatments for ovarian MLAs.

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CRediT authorship contribution statement

Gwan Hee Han: Conceptualization, Data curation, Formal analysis, Funding acquisition, Resources, Visualization, Writing – original draft. **Ye-Ah Kim:** Methodology, Visualization, Writing – original draft. **Hyunjin Park:** Visualization, Writing – original draft. **Hee Yun:** Conceptualization, Methodology, Validation, Writing – review & editing. **Jae-Hoon Kim:** Conceptualization, Resources, Writing – review & editing. **Man S. Kim:** Data curation, Formal analysis, Methodology, Software, Writing – review & editing. **Hanbyoul Cho:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gore.2024.101670.

References

Belloni, D., Scabini, S., Foglieni, C., Veschini, L., Giazzon, A., Colombo, B., et al., 2007. The vasostatin-I fragment of chromogranin A inhibits VEGF-induced endothelial cell proliferation and migration. The FASEB Journal. 21 (12), 3052–3062.

Castillo, P.E., Younts, T.J., Chávez, A.E., Hashimotodani, Y., 2012. Endocannabinoid signaling and synaptic function. Neuron. 76 (1), 70–81.

Crippa, L., Bianco, M., Colombo, B., Gasparri, A.M., Ferrero, E., Loh, Y.P., et al., 2013. A new chromogranin A–dependent angiogenic switch activated by thrombin. Blood, the Journal of the American Society of Hematology. 121 (2), 392–402.

Deolet, E., Van Dorpe, J., Van de Vijver, K., 2021. Mesonephric-like adenocarcinoma of the endometrium: diagnostic advances to spot this wolf in sheep's clothing. A review of the literature. Journal of. Clinical Medicine. 10 (4), 698.

Fang, L., Li, T.-S., Zhang, J.-Z., Liu, Z.-H., Yang, J., Wang, B.-H., et al., 2021. Fructose drives mitochondrial metabolic reprogramming in podocytes via Hmgcs2-stimulated fatty acid degradation. Signal Transduction and Targeted Therapy. 6 (1), 253.

Garlich, J.R., De, P., Dey, N., Su, J.D., Peng, X., Miller, A., et al., 2008. A vascular targeted pan phosphoinositide 3-kinase inhibitor prodrug, SF1126, with antitumor and antiangiogenic activity. Cancer Res. 68 (1), 206–215.

Grimm, D., Bauer, J., Wise, P., Krüger, M., Simonsen, U., Wehland, M., et al., 2020. The role of SOX family members in solid tumours and metastasis. Semin Cancer Biol. 67 (Pt 1), 122–153.

Jia, D., Park, J.H., Jung, K.H., Levine, H., Kaipparettu, B.A., 2018. Elucidating the Metabolic Plasticity of Cancer: Mitochondrial Reprogramming and Hybrid Metabolic States. Cells. 7 (3).

Jia, M., Sun, P.-L., Gao, H., 2019. Uterine lesions with sex cord-like architectures: a systematic review. Diagnostic Pathology. 14 (1), 1–10.

Kim, H.G., Kim, H., Yeo, M.K., Won, K.Y., Kim, Y.S., Han, G.H., et al., 2022. Mesonephric-like Adenocarcinoma of the Uterine Corpus: Comprehensive Analyses of Clinicopathological, Molecular, and Prognostic Characteristics With Retrospective Review of 237 Endometrial Carcinoma Cases. Cancer Genomics Proteomics. 19 (4), 526–539.

Koh, H.H., Park, E., Kim, H.S., 2022. Mesonephric-like Adenocarcinoma of the Ovary: Clinicopathological and Molecular Characteristics. Diagnostics (basel). 12 (2).

Kolberg, L., Raudvere, U., Kuzmin, I., Adler, P., Vilo, J., Peterson, H., 2023. g:Profiler-interoperable web service for functional enrichment analysis and gene identifier mapping (2023 update). Nucleic Acids Res. 51 (W1), W207–W212.

Korotkevich G, Sukhov V, Budin N, Shpak B, Artyomov MN, Sergushichev A. 2021. Fast gene set enrichment analysis. bioRxiv. 060012.

Li, J., Li, M.-h., Wang, T.-t., Liu, X.-n., Zhu, X.-t., Dai, Y.-z., et al., 2021. SLC38A4 functions as a tumour suppressor in hepatocellular carcinoma through modulating Wnt/β-catenin/MYC/HMGCS2 axis. British Journal of Cancer. 125 (6), 865–876.

Lin, D.I., Shah, N., Tse, J.Y., Killian, J.K., Hemmerich, A., Edgerly, C., et al., 2020. Molecular profiling of mesonephric and mesonephric-like carcinomas of cervical, endometrial and ovarian origin. Gynecol Oncol Rep. 34, 100652.

Lundgren, S., Berntsson, J., Nodin, B., Micke, P., Jirström, K., 2016. Prognostic impact of tumour-associated B cells and plasma cells in epithelial ovarian cancer. Journal of Ovarian Research. 9 (1), 1–9.

Macpherson, A.M., Barry, S.C., Ricciardelli, C., Oehler, M.K., 2020. Epithelial ovarian cancer and the immune system: biology, interactions, challenges and potential advances for immunotherapy. J Clin Med. 9 (9).

McCluggage, W.G., 2022. Mesonephric-like adenocarcinoma of the female genital tract: from morphologic observations to a well-characterized carcinoma with aggressive clinical behavior. Advances in Anatomic Pathology. 29 (4), 208–216.

McCluggage WG, Singh N, Gilks CB. 2022. Key changes to the World Health Organization (WHO) classification of female genital tumours introduced in the 5th edition (2020). Histopathology. 80 (5), 762-78.

Mirkovic, J., Sholl, L.M., Garcia, E., Lindeman, N., MacConaill, L., Hirsch, M., et al., 2015. Targeted genomic profiling reveals recurrent KRAS mutations and gain of chromosome 1q in mesonephric carcinomas of the female genital tract. Modern Pathology. 28 (11), 1504–1514.

Mirkovic, J., Olkhov-Mitsel, E., Amemiya, Y., Al-Hussaini, M., Nofech-Mozes, S., Djordjevic, B., et al., 2023. Mesonephric-like adenocarcinoma of the female genital tract: novel observations and detailed molecular characterisation of mixed tumours and mesonephric-like carcinosarcomas. Histopathology.

Na, K., Kim, H.-S., 2019. Clinicopathologic and molecular characteristics of mesonephric adenocarcinoma arising from the uterine body. The American Journal of Surgical Pathology. 43 (1), 12–25.

Nath, A., Cosgrove, P.A., Mirsafian, H., Christie, E.L., Pflieger, L., Copeland, B., et al., 2021. Evolution of core archetypal phenotypes in progressive high grade serous ovarian cancer. Nat Commun. 12 (1), 3039.

Pors, J., Segura, S., Chiu, D.S., Almadani, N., Ren, H., Fix, D.J., et al., 2021. Clinicopathologic characteristics of mesonephric adenocarcinomas and mesonephric-like adenocarcinomas in the gynecologic tract: a multi-institutional study. The American Journal of Surgical Pathology. 45 (4), 498.

Qin, A.C., Li, Y., Zhou, L.N., Xing, C.G., Lu, X.S., 2019. Dual PI3K-BRD4 Inhibitor SF1126 Inhibits Colorectal Cancer Cell Growth in Vitro and in Vivo. Cell Physiol Biochem. 52 (4), 758–768.

Qiu, P., Chen, Y., Dong, C., Xie, J., Wang, J., 2024. Transcriptome characteristics of epithelial cells from advanced non-small cell lung cancer were revealed by single-cell RNA sequencing. Environmental Toxicology. 39 (2), 723–735.

Rath, S., Sharma, R., Gupta, R., Ast, T., Chan, C., Durham, T.J., et al., 2021. MitoCarta3.0: an updated mitochondrial proteome now with sub-organelle localization and pathway annotations. Nucleic Acids Res. 49 (D1), D1541–D1547.

Ray-Coquard, I., Pignata, S., Lee, J., Coleman, R., Brown, J., Kim, J., et al., 2023. 747MO First results from the ENGOT-GYN2/GOG-3051/BOUQUET phase II biomarker-directed platform study: Cobimetinib (cobi) or atezolizumab (atezo)+ bevacizumab (bev) for persistent/recurrent rare epithelial ovarian cancer (eOC). Annals of Oncology. 34, S511–S512.

Ren, Y., Li, R., Feng, H., Xie, J., Gao, L., Chu, S., et al., 2022. Single-cell sequencing reveals effects of chemotherapy on the immune landscape and TCR/BCR clonal expansion in a relapsed ovarian cancer patient. Front Immunol. 13, 985187.
Shen, Y., Ren, Y., Chen, K., Cen, Y., Zhang, B., Lu, W., et al., 2022. The impact of neoadjuvant chemotherapy on the tumor microenvironment in advanced high-grade serous carcinoma. Oncogenesis. 11 (1), 43.
Shih, A.J., Menzin, A., Whyte, J., Lovecchio, J., Liew, A., Khalili, H., et al., 2018. Identification of grade and origin specific cell populations in serous epithelial ovarian cancer by single cell RNA-seq. PLoS One. 13 (11), e0206785.
Stuart, T., Butler, A., Hoffman, P., Hafemeister, C., Papalexi, E., Mauck, W.M., et al., 2019. Comprehensive integration of single-cell data. Cell. 177 (7), 1888–902. e21.
Szklarczyk, D., Franceschini, A., Wyder, S., Forslund, K., Heller, D., Huerta-Cepas, J., et al., 2015. STRING v10: protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res. 43 (Database issue), D447–D452.
Tang, F., Barbacioru, C., Wang, Y., Nordman, E., Lee, C., Xu, N., et al., 2009. mRNA-Seq whole-transcriptome analysis of a single cell. Nature Methods. 6 (5), 377–382.
van der Mijn, J.C., Panka, D.J., Geissler, A.K., Verheul, H.M., Mier, J.W., 2016. Novel drugs that target the metabolic reprogramming in renal cell cancer. Cancer Metab. 4, 14.
Wan, C., Keany, M.P., Dong, H., Al-Alem, L.F., Pandya, U.M., Lazo, S., et al., 2021. Enhanced Efficacy of Simultaneous PD-1 and PD-L1 Immune Checkpoint Blockade in High-Grade Serous Ovarian Cancer. Cancer Res. 81 (1), 158–173.
Wang, Z., Yang, L., Su, X., Wu, X., Su, R., 2023. Single-cell RNA-sequencing analysis reveals divergent transcriptome events between platinum-sensitive and platinum-resistant high-grade serous ovarian carcinoma. J Gene Med. 25 (10), e3504.
Webb, P.M., Jordan, S.J., 2017. Epidemiology of epithelial ovarian cancer. Best Pract Res Clin Obstet Gynaecol. 41, 3–14.
Xu, J., Fang, Y., Chen, K., Li, S., Tang, S., Ren, Y., et al., 2022. Single-Cell RNA Sequencing Reveals the Tissue Architecture in Human High-Grade Serous Ovarian Cancer. Clin Cancer Res. 28 (16), 3590–3602.